INTRODUCTION TO BIOCHEMISTRY

Separation 00:03:29

- Separation of subcellular organelles from a given tissue is based on the principle called ultra-centrifugation

Procedure

Tissue sample

↓

Homogenization (crush the tissue so that sub-cellular organelles come into mixture

↓

Homogenized tissue mixture/ suspension

↓

Ultra-centrifugation at 1000g for 10 minutes

Pellet

(on examination)

- Intact/ whole cells
- Nuclei
- Cytoskeleton
- Plasma membrane

Supernatant

↓

Ultra-centrifugation at 20,000 for 20 minutes

Pellet

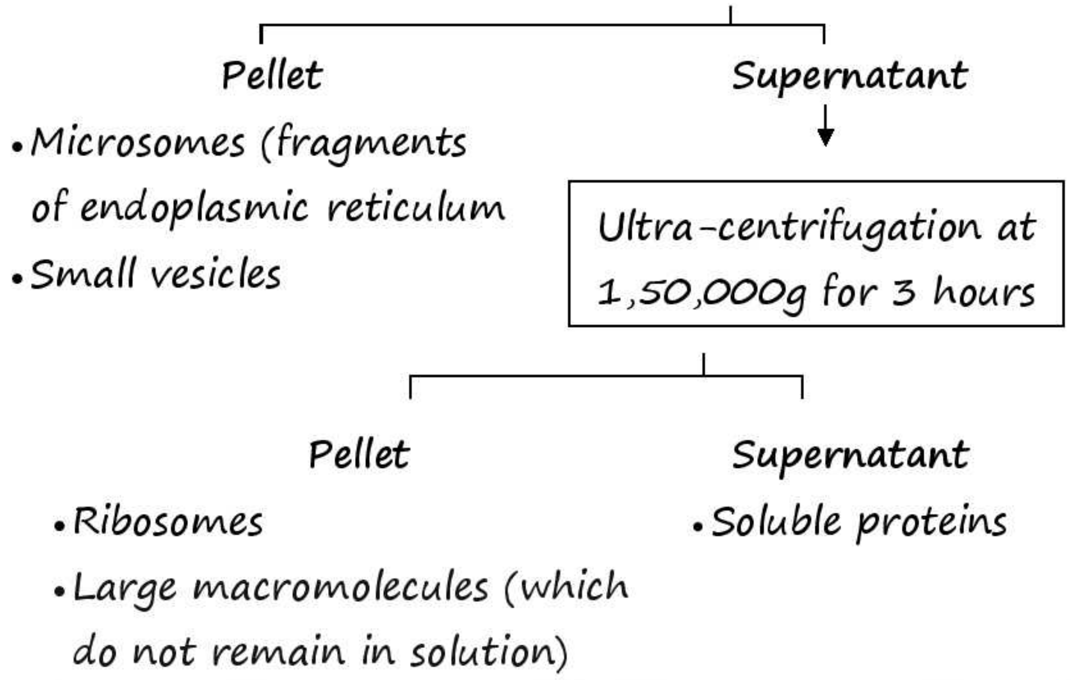
- Mitochondria
- Lysosomes
- Peroxisomes

Supernatant

↓

Ultra-centrifugation at 80,000g for 1 hour

DOT TUTORIALS
LOGIN FOR SUCCESS



Pellet

- Microsomes (fragments of endoplasmic reticulum
- Small vesicles

Supernatant

Ultra-centrifugation at 1,50,000g for 3 hours

Pellet

- Ribosomes
- Large macromolecules (which do not remain in solution)

Supernatant

- Soluble proteins

- Note: Sedimentation depends on sedimentation coefficient (s) (or svedberg coefficient)

Identification 00:12:34

- Identification of sub-cellular organelles is done by markers

Sub-cellular organelle	Marker
Cytoplasm	Lactate dehydrogenase
Mitochondria (Inner mitochondrial membrane)	ATP synthase/ F_0F_1 particle/ Complex V of electron transport chain
Endoplasmic reticulum/ microsomes	Glucose-6-phosphatase (liver, kidney)
Lysosomes	Cathepsin
Golgi body	Galactosyl transferase
Peroxisome	Catalase, urate oxidase
Plasma membrane	Na^{+}-K^{+} ATPase, Adenylyl cyclase, S' nucleotidase

- Note: Lactate dehydrogenase
  - Types I, II, III, IV, V
  - I – Heart
  - II – RBC (Red Blood Cells)
  - III – Non-specific
  - IV } Liver and muscles
  - V

- Outer mitochondrial membrane is similar to plasma membrane hence inner membrane marker is considered for identification of mitochondria

Different metabolic pathways according to cellular compartments 00:18:19

Cellular compartment	Metabolic pathways
Cytoplasm	Glycolysis HMP shunt Glycogenesis Purine and pyrimidine synthesis Lipogenesis Cholesterol synthesis Urea cycle } Partly Gluconeogenesis } Partly
Mitochondria	Kreb's/ TCA cycle

	Electron Transport chain/ Respiratory chain Ketogenesis β-oxidation Urea cycle Gluconeogenesis } Partly
Nucleus	DNA synthesis (DNA replication in 'S' phase) RNA synthesis (Transcription) Regulation of gene expression
Endoplasmic reticulum	Translation (protein synthesis) Fatty acid elongation Omega-oxidation of fatty acids Post translational modification of proteins Alcohol metabolism
Peroxisomes	α-oxidation (metabolism of branched fatty acid) Oxidation of VLCFA (Very Long Chain Fatty Acid)

Newer sub-specializations in biochemistry

00:24:57

- Genome (complete sequence of nucleotides in a cell)
  - Genomics (study of genome)
- Gene (precursor of genome)

  - Genetics (study of genes)
- Proteome (complete protein content of a cell)
  - Proteomics (study of proteome)
- Glycome (complete carbohydrate content of a cell)
  - Glycomics (study of glycome)
- Lipodome (complete lipid content of a cell)
  - Lipidomics (study of lipidome)
- Metabolome (complete set of metabolites in a cell)
  - Metabolomics (study of metabolome)
- Epigenome (chemical modification of DNA or DNA associated protein, that alter gene expression) for example – without changing the sequence of nucleotides, covalent modification of histones alter rate of expression i.e., transcription
  - Epigenomics/ Epigenetics (study of epigenome)

Correlation of Biochemistry with medicine

00:30:10

DOC TUTORIALS LOGIN FOR SUCCESS

- Presently evidence-based medicine is being practiced
- 80-85% of data regarding the evidence come from biochemistry lab

Other uses of biochemistry

- Inborn errors of metabolism
- Oncogenes
- Loss of tumor suppressor genes

- Biochemical basis of some chronic diseases
  - Hypertension
  - Diabetes
  - Polycystic ovarian Disease (PCOD)

ACID BASE HOMEOSTASIS

pH review

- $pH = -\log[H^+]$
- Range 0 to 14 (according to chemical lab)
- If (H^+) is high, solution is acidic, $pH<7$
- If (H^+) is low, solution is basic, $pH>7$
- $(H^+) \propto 1/(OH^-)$
- Neutral $pH=7.0$
- Interpretation of pH in terms of concentration of H^+ ions

Grams of H^+ per Liter	pH	
0.00000000000001	14	↑
0.0000000000001	13	
0.000000000001	12	
0.00000000001	11	
0.0000000001	10	acidic Increasing basic
0.000000001	9	
0.00000001	8	Neutral-neither acidic nor basic
0.0000001	7	
0.000001	6	
0.00001	5	Increasingly
0.0001	4	
0.001	3	↓
0.01	2	
0.1	1	
1.0	0	

TUTORIALS LOGIN FOR SUCCESS

- Acids are H^+ donors
- Bases are H^+ acceptors or give OH^- in solution
- Acid and base can be
  - Strong → dissociate completely in solution (irrespective of pH) – Ex: HCl, NaOH
  - Weak → only partially dissociate in solution (dependent on pH) – Ex: Lactic acid, carbonic acid

Body and pH 00:04:32

- Homeostasis of pH is tightly controlled
- Extracellular fluid pH=7.4
- Blood pH = 7.35 – 7.45
- Survival range = 6.8 – 8.0 (<6.8 or >8.0 – death occurs)

- Acidosis / Acidemia: < 7.35
- Alkalosis / Alkalemia: > 7.45

TUTORIALS LOGIN FOR SUCCESS

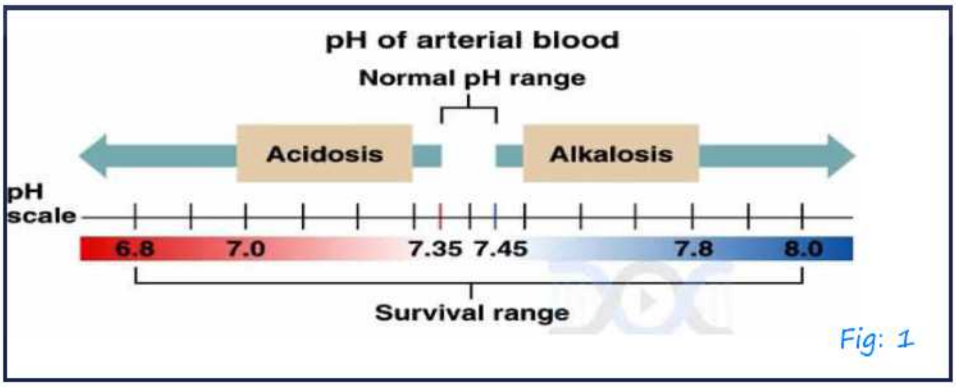

pH of arterial blood
Normal pH range
Acidosis
Alkalosis
pH scale
6.8
7.0
7.35
7.45
7.8
8.0
Survival range

Fig: 1

- Small changes in pH can produce major disturbances
- Most enzymes function only within narrow pH range (optimum pH)
- Acid base balance can also affect electrolytes (Na^+, K^+, Cl^-)

Body produces more acids than bases

- Acids taken in with foods
- Acids produced by metabolism of lipids/ proteins /carbohydrates

↓ ↓ ↓

Fatty acids aminoacids lactic acid

- Cellular metabolism produces Co_2
- $Co_2 + H_2o \leftrightarrow H_2Co_3 \leftrightarrow H^+ + HCo_3^-$

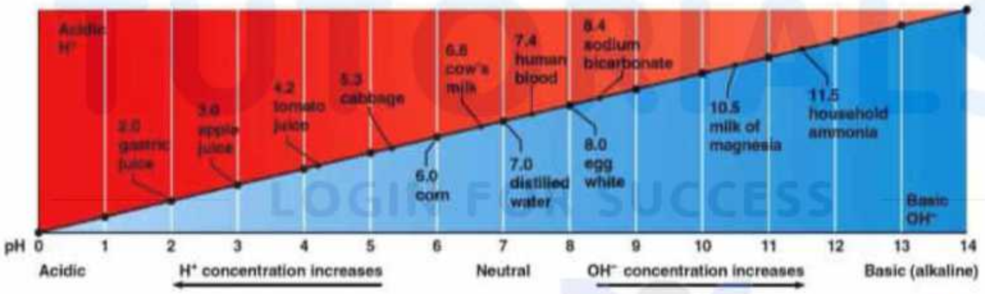


Fig: 2

- Then, how is pH controlled?
- It is by buffer systems, respiratory system and renal system

Buffer's systems

- Buffers are solutions that resist a small change in pH
- Buffers contain relatively large concentrations of either
  - An acid (HA) and it's conjugate base A^-
  - A base (B) and it's conjugate base BH^+
- Exchanges a strong acid/base for a weak acid/base
- Buffers result in a much smaller pH change
- Buffers are supported by respiratory and renal mechanisms

Respiratory mechanism

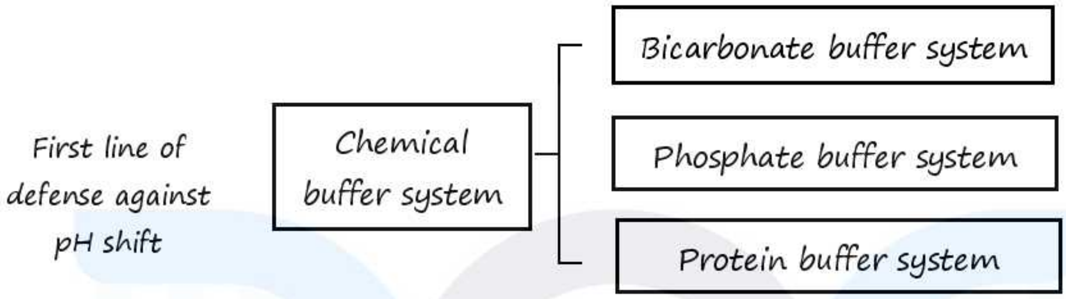
- Exhalation of Co_2
- Powerful, but only works for volatile acids
- Doesn't affect fixed acids like lactic acid
- Body pH can be adjusted by changing the rate and depth of breathing

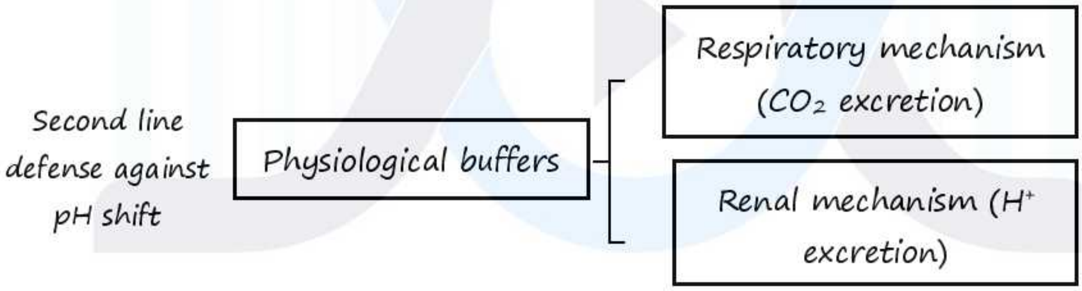
Renal mechanism

- Can eliminate large amounts of acid (H^+ buffered and eliminated in urine)
- Can also excrete base
- Can conserve and produce bicarbonate ions
- Most effective regulator of pH
- If kidneys fail, pH balance fails

Rates of correction

- Buffers function almost instantaneously
- Respiratory mechanism – takes several minutes to hours
- Renal mechanism – takes several hours to days


First line of defense against pH shift
Chemical buffer system
Bicarbonate buffer system
Phosphate buffer system
Protein buffer system


Second line defense against pH shift
Physiological buffers
Respiratory mechanism (CO_2 excretion)
Renal mechanism (H^+ excretion)

- Buffer function is described by Henderson-Hasselbalch equation
- The equation describes the pH at which buffer is going to act or pH which can be achieved by buffer

  pH = pK + log (Base)/ (Acid), where (pK = dissociation constant)

  (or)

  pH = pK – log (Acid)/ (Base)

- Example: Bicarbonate buffer system

  $pH = pK + \log [HCO_3^-] / [H_2CO_3]$

- Buffer capacity → amount of acid/base required to change the pH by one unit
  - Depends on concentration of analytes
- Buffering action → best: when target pH = pK
- Optimum: when pH = pK ± 1

Buffer system

- Bicarbonate buffer system
- Phosphate buffer system
- Plasma protein buffer system
- Hemoglobin buffer system

TUTORIALS
LOOK FOR SUCCESS

Bicarbonate/ carbonic acid

- $pH = pK + \log \frac{[HCO_3^-]}{[H_2CO_3]}$ → Base (bicarbonate ion)
- Most important buffer system
- $pK = 6.1$
- Best buffer system due to:
  - Good buffering action (due to exact target pH)
  - Infinite buffering capacity

Buffering action

- Optimum range of buffering action → 5.1 to 7.1
- But target pH at which it acts → 7.4

- How is target pH achieved?
  - The ratio of base to acid in body → 20:1
  - According to Henderson – Hasselbalch equation

    $pH = 6.1 + 1.3$ $(\log 20 = 1.3)$

    $= 7.4$

Buffer capacity

- Lungs → readily dispose/ retain CO_2
- Renal tubules → increase/decrease rate of bicarbonate reclamation
- Buffer capacity infinite (∞) because the concentration of numerator and denominator can be changed as much as needed

Phosphate buffer system 00:26:34

- $pH = pK + \log \dfrac{(HPO_4^{2-}) \rightarrow \text{base}}{(H_2PO_4^{-}) \rightarrow \text{acid}}$
- $pK = 6.8$
- Intracellular fluid pH = 7.0
- Renal tubular fluid pH = 6 to 6.5
- 5% of non-bicarbonate buffer value of plasma

So, phosphate is good buffer for these because target pH is close to pK

Important buffer in

Renal tubular fluid because of target pH and high concentration of phosphate as filtered phosphate can't be reabsorbed

Intracellular fluid because of target pH and the concentration of phosphate is many times than in extracellular fluid

Plasma protein buffer system 00:26:34

- Albumin – 95% of non-bicarbonate buffer value of plasma
  - Has large amounts of histidine
- Imidazole group of histidine – pK = 7.3

AfroTafreeh.com

Hemoglobin buffer system 00:27:31

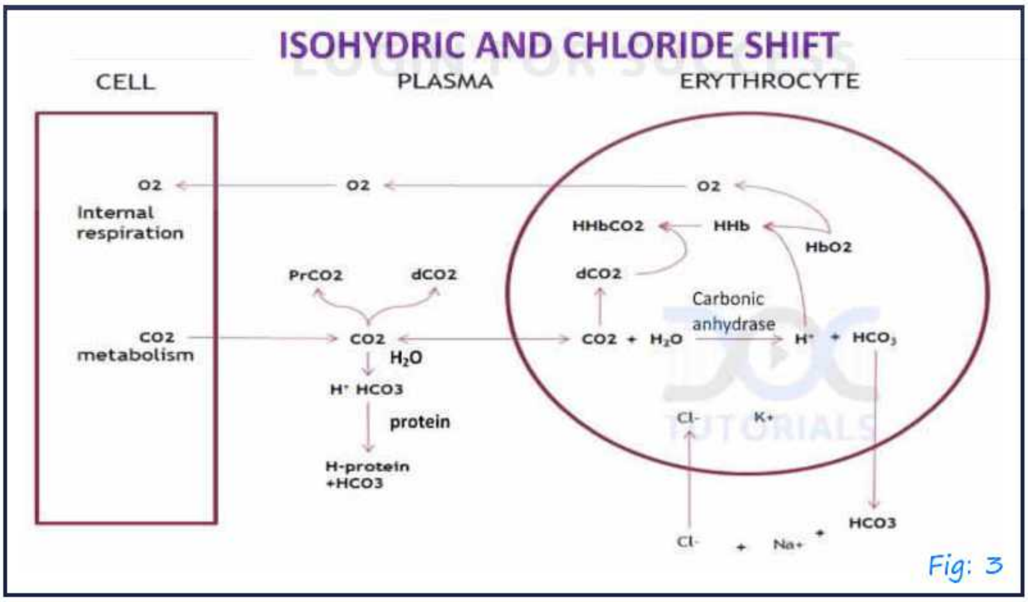

ISOHYDRIC AND CHLORIDE SHIFT
CELL
PLASMA
ERYTHROCYTE
O2
Internal respiration
CO2 metabolism
O2
PrCO2
dCO2
CO2
H2O
H+ HCO3
protein
H-protein +HCO3
O2
HHbCO2
HHb
HbO2
dCO2
Carbonic anhydrase
CO2 + H2O
H+ + HCO3
Cl-
K+
HCO3
Cl- + Na+

Fig: 3

- Note: Carbonic anhydrase speeds up the reactions to 10^8 times
- Dual buffering action of hemoglobin:
  - $HbO_2 + H^+$
  - $HHb + dCO_2$

HbO_2 = Oxygenated hemoglobin

HHb = Proteinated hemoglobin

dCO_2 = Dissolved carbon dioxide

Respiratory mechanism

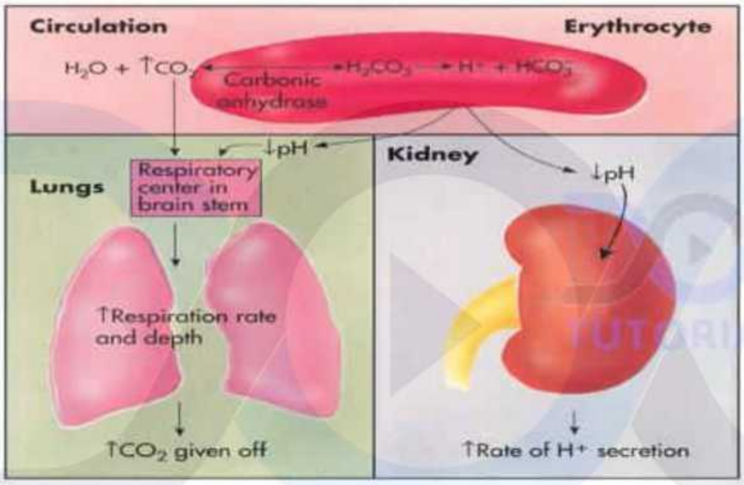

Circulation
Erythrocyte
H₂O + ↑CO₂
Carbonic anhydrase
H₂CO₃
H⁺ + HCO₃⁻
↓pH
Respiratory center in brain stem
Lungs
↑Respiration rate and depth
↑CO₂ given off
Kidney
↓pH
↑Rate of H⁺ secretion

Fig: 4

- 3 ways
  - Reclamation of bicarbonate
  - Hydrogen ion excretion
  - Renal production of ammonia and excretion of ammonium ions

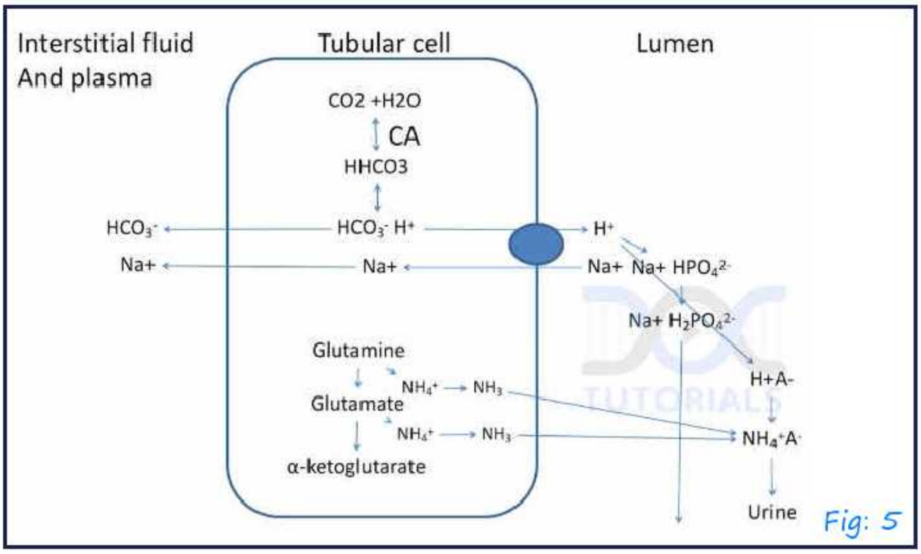

Interstitial fluid
And plasma
Tubular cell
Lumen
CO2 +H2O
CA
HHCO3
HCO3-
HCO3- H+
H+
Na+
Na+
Na+ Na+ HPO4 2-
Na+ H2PO4 2-
Glutamine
NH4+ → NH3
Glutamate
NH4+ → NH3
α-ketoglutarate
H+A-
NH4+A-
Urine

Fig: 5

- Glutamine $\xrightarrow{\text{Glutaminase}}$ Glutamate $\xrightarrow{\text{Glutamate dehydrogenase}}$ α Ketoglutarate
- The filtered Na^+ and HCO_3^- both are reabsorbed
- Excretion of H^+ via phosphate

ACID BASE DISORDERS

- pH < 7.35 → Acidosis
- pH > 7.45 → Alkalosis
- The body response to acid base imbalance is called compensation
- Complete compensation if pH brought back within normal limits
- Partial compensation if pH is still outside normal limit

Compensation

If underlying problem is metabolic (renal)
↓
Respiratory compensation (hyper/ hypoventilation)

If underlying problem is respiratory
↓
Renal/ Metabolic compensation

Acidosis

- Principle effect of acidosis → depression of CNS through decrease in synaptic transmission (headache, lethargy)
- Generalized weakness, nausea, vomiting, diarrhea
- Deranged CNS function – greatest threat
- Severe acidosis causes
  - Disorientation
  - Coma
  - Death

Alkalosis

- Causes over excitability of CNS and PNS
- Numbness
- Lightheadness
- Nervousness
- Muscles spasms & tetany
- Convulsions
- Loss of consciousness
- Respiration – slow and shallow
- Hyperactive reflexes
- Atrial tachycardia
- Dysrhythmias
- Depletion of electrolytes
- Death

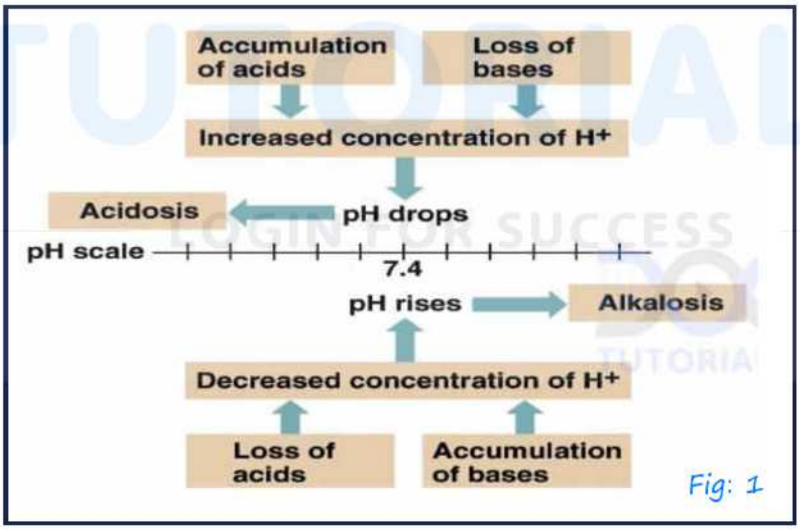

Accumulation of acids
Loss of bases
Increased concentration of H+
Acidosis
pH drops
pH scale
7.4
pH rises
Alkalosis
Decreased concentration of H+
Loss of acids
Accumulation of bases

Fig: 1

Acid Base disorders 00:04:11

- Normal pH – 7.4 (7.35 to 7.45)
- Normal $[HCO_3^-]$ – 24 mEq/L (22 to 26 mEq/L)
- PCO_2 – 40mmHg (35 to 45 mmHg)

	Metabolic	Respiratory
Acidosis	Decrease [HCO_3^-]	Increase pCO_2
Alkalosis	Increase [HCO_3^-]	Decrease pCO_2

Metabolic acidosis 00:07:48

- Bicarbonate deficit – blood concentration of bicarbonate → <22mEq/L
- Causes
  - Excessive production of acids (ketoacidosis) → Anion gap increases
  - Decreased excretion of acids (Renal Tubular Acidosis)
  - Excessive loss of bicarbonate (diarrhoea)

Anion gap

- Anion gap for sodium
- $Na^+ - (Cl^- + HCO_3^-) = 12$ mmol/L
- Anion gap for sodium and potassium
- $(Na^+ + K^+) - (Cl^- + HCO_3^-) = 8$ mmol/L
- Anion gap: – difference between measured cations and measured anions

↓ Na^+, K^+ ↓ Cl^-, HCO_3^-

In case of excessive production of acids (metabolic acidosis)

↓

Different anions come into play

↓

Relative concentration of Cl^- and HCO_3^- falls

TUTORIALS LOGIN FOR SUCCESS

↓

Anion gap increases

Conditions in which anion gap increases

- M – Methanol poisoning
- U – Uremia
- D – Diabetic ketoacidosis, ketoacidosis due to excess alcohol intake or starvation
- P – Paraldehyde
- I – Isoniazid, Iron
- L – Lactic acid
- E – Ethanol, Ethylene glycol
- S – Salicylates

Compensation for metabolic acidosis

- Respiratory mechanism
    - In acidosis $pH = pK + \log \dfrac{HCO_3^-}{(0.03 \times pCO_2)}$ (decreased)
    - By increasing ventilation – pCO_2 decreases
- Renal mechanism
    - Increase Na^+ H^- exchange
    - Increase formation of ammonia
    - Increase reclamation of bicarbonate

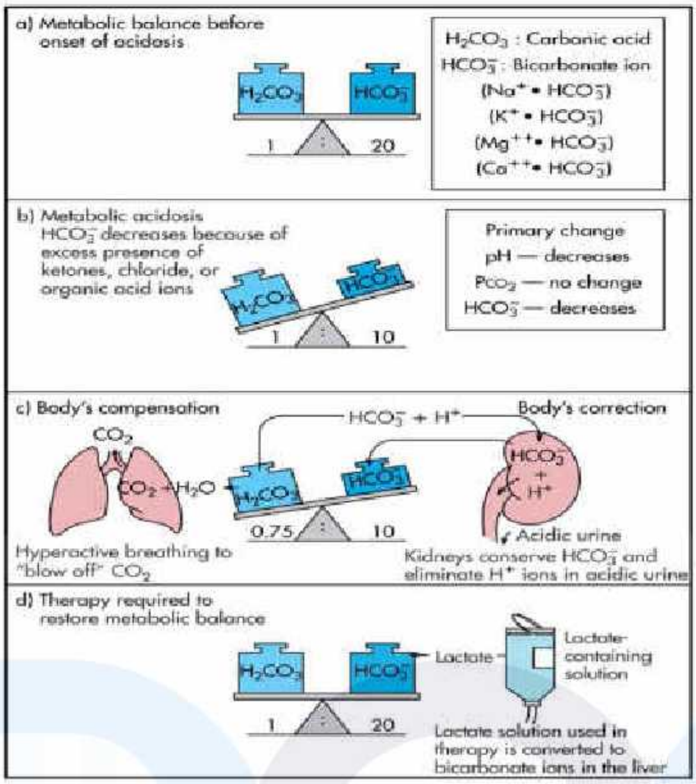

a) Metabolic balance before onset of acidosis
H₂CO₃
HCO₃⁻
1
20
H₂CO₃ : Carbonic acid
HCO₃⁻ : Bicarbonate ion
(Na⁺ • HCO₃⁻)
(K⁺ • HCO₃⁻)
(Mg⁺⁺ • HCO₃⁻)
(Ca⁺⁺ • HCO₃⁻)
b) Metabolic acidosis
HCO₃⁻ decreases because of excess presence of ketones, chloride, or organic acid ions
1
10
Primary change
pH — decreases
Pco₂ — no change
HCO₃⁻ — decreases
c) Body's compensation
CO₂
CO₂ + H₂O
HCO₃⁻ + H⁺
Body's correction
0.75
10
HCO₃⁻ + H⁺
Acidic urine
Hyperactive breathing to "blow off" CO₂
Kidneys conserve HCO₃⁻ and eliminate H⁺ ions in acidic urine
d) Therapy required to restore metabolic balance
Lactate
Lactate-containing solution
1
20
Lactate solution used in therapy is converted to bicarbonate ions in the liver

Fig: 2

Metabolic alkalosis 00:17:36

- Bicarbonate excess – concentration in blood > 26 mEq/L
- Causes:
  - Excess vomiting → loss of stomach acid i.e., H^+
  - Excess use of alkaline drugs
  - Certain diuretics

Compensation for metabolic acidosis

- Respiratory mechanism
  - In acidosis $$pH = pK + \log \frac{HCO_3^- \text{ (increased)}}{(0.03 \times pCO_2)}$$
  - By retention – pCO_2 increase
- Renal mechanism

- Decrease Na^+ H^- exchange
- Decrease formation of ammonia
- Decrease reclamation of bicarbonate

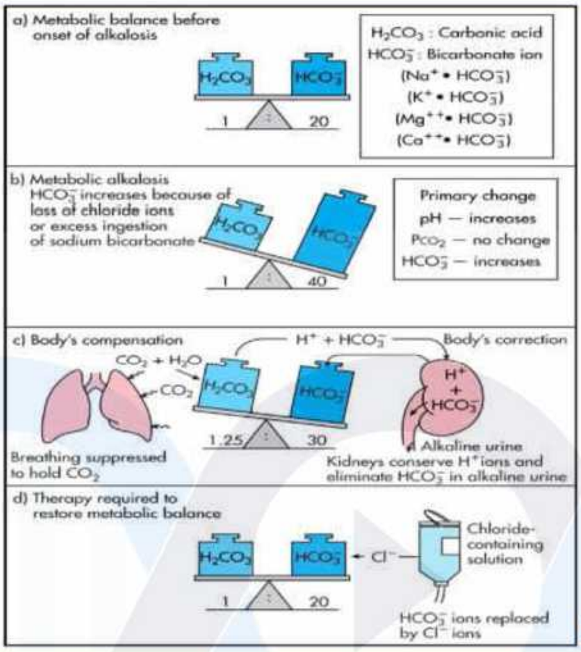

a) Metabolic balance before onset of alkalosis
H_2CO_3
HCO_3^-
1
20
H_2CO_3 : Carbonic acid
HCO_3^- : Bicarbonate ion
$(Na^+ \bullet HCO_3^-)$
$(K^+ \bullet HCO_3^-)$
$(Mg^{++} \bullet HCO_3^-)$
$(Ca^{++} \bullet HCO_3^-)$
b) Metabolic alkalosis
HCO_3^- increases because of loss of chloride ions or excess ingestion of sodium bicarbonate
H_2CO_3
HCO_3^-
1
40
Primary change
pH — increases
P_{CO_2} — no change
HCO_3^- — increases
c) Body's compensation
$H^+ + HCO_3^-$
Body's correction
$CO_2 + H_2O$
CO_2
H_2CO_3
HCO_3^-
1.25
30
H^+ + HCO_3^-
Alkaline urine
Breathing suppressed to hold CO_2
Kidneys conserve H^+ ions and eliminate HCO_3^- in alkaline urine
d) Therapy required to restore metabolic balance
H_2CO_3
HCO_3^-
Cl^-
1
20
Chloride-containing solution
HCO_3^- ions replaced by Cl^- ions

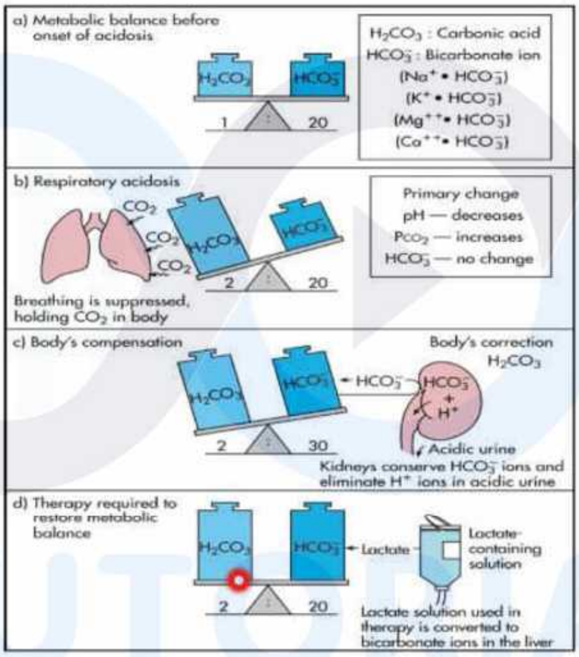
Fig:3

Respiratory acidosis 00:20:12

- Any condition that decreases the elimination of CO_2 (pCO_2 >40mmHg)
- Causes:
  - Respiratory centre depressed (trauma/ tumor/ Infection/ Drugs)
  - Respiratory apparatus affected (COPD/fibrosis/ARDS/Trauma)
- Signs and symptoms;
  - Breathlessness, restlessness, lethargy, disorientation, tremors, convulsions, coma, Respiratory rate then gradually depressed, skin warm and flushed due to vasodilation caused by excess CO_2

Compensation for metabolic acidosis

- Buffer systems
  - Hemoglobin and protein buffers
- Renal mechanism
  - Increase Na^+ H^- exchange
  - Increase formation of ammonia
  - Increase reclamation of bicarbonate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($K^+ \bullet HCO_3^-$)
($Mg^{++} \bullet HCO_3^-$)
($Ca^{++} \bullet HCO_3^-$)
b) Respiratory acidosis
CO_2
CO_2
CO_2
H_2CO_3
HCO_3^-
2
20
Breathing is suppressed, holding CO_2 in body
Primary change
pH — decreases
P_{CO_2} — increases
HCO_3^- — no change
c) Body's compensation
H_2CO_3
HCO_3^-
2
30
Body's correction
H_2CO_3
HCO_3^-
HCO_3^-
+
H^+
Acidic urine
Kidneys conserve HCO_3^- ions and eliminate H^+ ions in acidic urine
d) Therapy required to restore metabolic balance
H_2CO_3
HCO_3^-
2
20
Lactate
Lactate-containing solution
Lactate solution used in therapy is converted to bicarbonate ions in the liver

Fig:4

DCI TUTORIALS LOGIN FOR SUCCESS

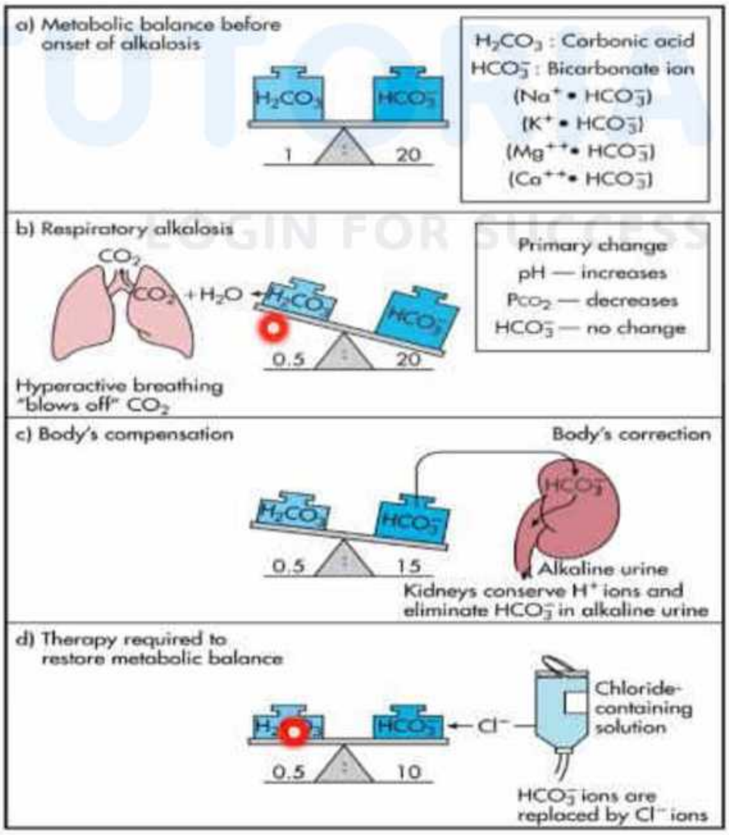
Respiratory alkalosis

- Carbonic acid deficit
- $pCO_2 < 35mmHg$ (hypocapnia)
- Most common acid base imbalance
- Primary cause → hyperventilation
- Conditions that stimulate respiratory centre
  - Oxygen deficiency at high altitudes
  - Pulmonary disease and congestive heart failure (caused by hypoxia)

- Acute anxiety
- Fever, anemia
- Salicylate intoxication
- Cirrhosis
- Gram negative sepsis
- Severe exercise

Compensation for metabolic acidosis

- Buffer systems
    - Hemglobin and protein buffers
- Renal mechanism
    - Decrease Na^+ H^- exchange
    - Decrease formation of ammonia
    - Decrease reclamation of bicarbonate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$(Na^+ \bullet HCO_3^-)$
$(K^+ \bullet HCO_3^-)$
$(Mg^{++} \bullet HCO_3^-)$
$(Ca^{++} \bullet HCO_3^-)$
b) Respiratory alkalosis
CO_2
$CO_2 + H_2O$
H_2CO_3
HCO_3^-
0.5
20
Hyperactive breathing "blows off" CO_2
Primary change
pH — increases
P_{CO_2} — decreases
HCO_3^- — no change
c) Body's compensation
Body's correction
H_2CO_3
HCO_3^-
0.5
15
HCO_3^-
Alkaline urine
Kidneys conserve H^+ ions and eliminate HCO_3^- in alkaline urine
d) Therapy required to restore metabolic balance
HCO_3^-
Cl^-
Chloride-containing solution
0.5
10
HCO_3^- ions are replaced by Cl^- ions

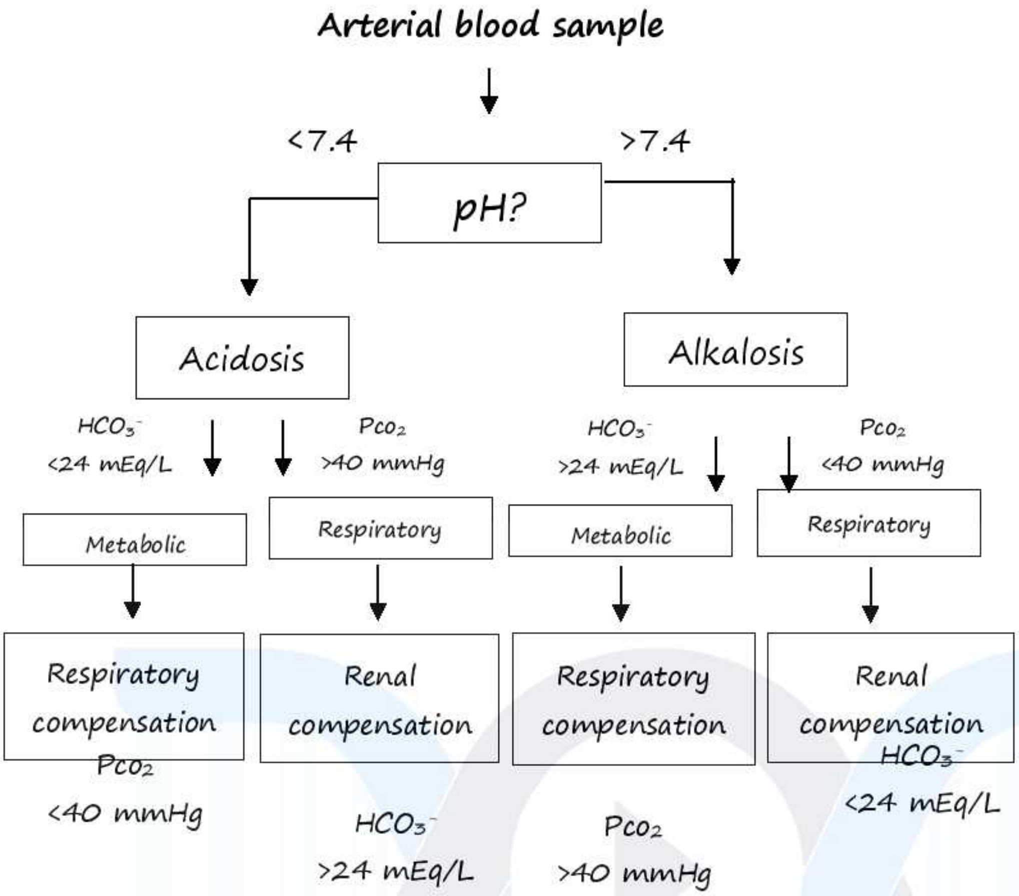
Fig:5

- Note:
  - Fluid of choice for correcting acidosis → Ringer lactate
  - Fluid of choice for correcting alkalosis → Normal saline

Diagnosis of acid base imbalances 00:25:12

- Note pH
  - Low → acidosis
  - High → alkalosis
- Decide which value, pCO_2 or (HCO_3^-) is outside the normal range and it could be the cause of the imbalance
  - If the cause is change in pCO_2 → problem is respiratory
  - If the cause is change in (HCO_3^-) → problem is metabolic
- Look at the value that doesn't correspond to observed pH change
  - If it is within normal range → no compensation **(acute cases)**
  - If it is outside normal range → partial compensation **(chronic cases)**

DOC TUTORIALS LOGIN FOR SUCCESS


Arterial blood sample
<7.4
>7.4
pH?
Acidosis
Alkalosis
HCO₃⁻ <24 mEq/L
Pco₂ >40 mmHg
HCO₃⁻ >24 mEq/L
Pco₂ <40 mmHg
Metabolic
Respiratory
Metabolic
Respiratory
Respiratory compensation
Renal compensation
Respiratory compensation
Renal compensation
Pco₂ <40 mmHg
HCO₃⁻ >24 mEq/L
Pco₂ >40 mmHg
HCO₃⁻ <24 mEq/L

Davenport diagram

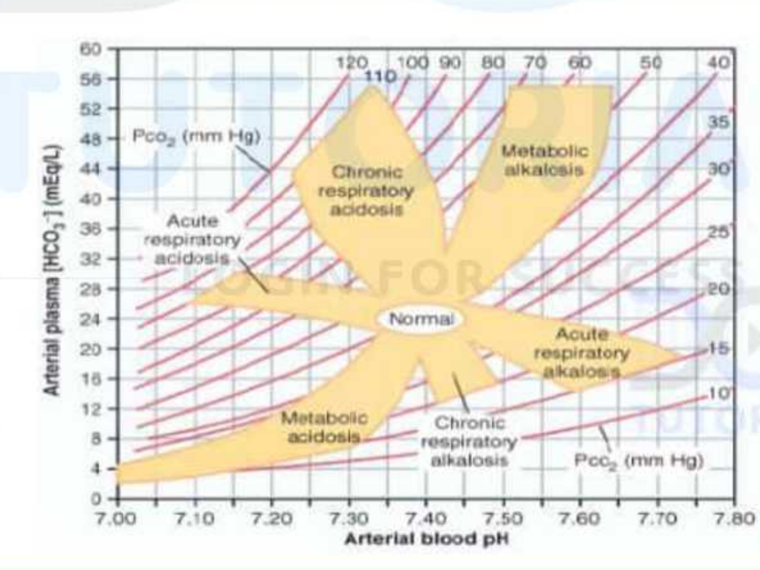


Fig:6

- Used in cases of emergency
- Q. A patient in ICU because of severe myocardial infarction 3 days ago. Lab report of ABG given below

- pH – 7.3
- HCO_3 – 20 mEq/L
- pCO_2 – 32 mmHg
- Ans. Metabolic acidosis with respiratory compensation
- Q. A lethargic presented to emergency with history of 3 episodes vomiting after excessive alcohol intake
- His ABG analysis shows → pH=7.6
- Ans. Metabolic acidosis → due to alcohol intake

  ⊕

  Metabolic alkalosis → due to vomiting

  ⊕

  Respiratory alkalosis → due to hyperventilation

  pH = 7.36

- Measure anion gap – increase (due to metabolic acidosis)

↓

Once anion gap corrected

↓

Alkalosis presents

↓

Treat the alkalosis

ACTIONS OF INSULIN

Fed – Fasting state 00:01:31

- Fed state – ↑ glucose (Due to Increase absorption)

↓

↑ Insulin (Due to stimulation of insulin release by glucose)

- Fasting state: ↓ Glucose

↓

↓ Insulin

Insulin – Glucagon ratio 00:03:59

$$\frac{\text{Insulin (I)}}{\text{Glucagon (G)}}$$

- Fed state → $\frac{I \uparrow}{G \downarrow}$ → Ratio ↑
- Fasting state → $\frac{I \downarrow}{G \uparrow}$ → Ratio ↓

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- Note – Simply the ↑ or ↓ in I/G ratio can be considered as ↑ or ↓ insulin respectively

Phosphorylated and Dephosphorylated Sate

00:07:15

- Insulin causes dephosphorylation of enzymes (almost always)
- Pathways
  - i. Activated by I (insulin) → Enzymes are activated in dephosphorylation form
  - ii. Inactivated by I (Insulin) → Enzymes are inactivated in dephosphorylation form

Actions of insulin

00:08:47

i. Consumes glucose

ii. Prevents formation of any new metabolic fuel (Glucose/Ketone bodies fatty acids)

Insulin

Upregulates

(Pathways that consume glucose)

- Glycolysis
- Glycogenesis
- Lipogenesis
- HMP shunt
- Amino acid synthesis
- Cholesterol synthesis

↓

Enzymes of these pathways are active in dephosphorylated state

Downregulates

(Pathways that synthesis metabolic fuels)

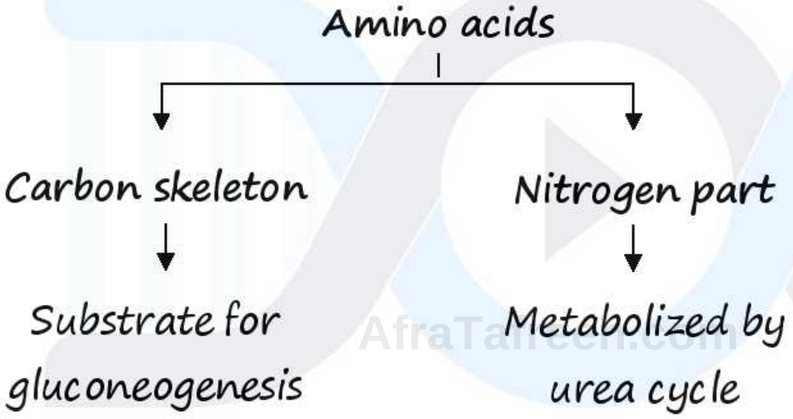
- Gluconeogenesis
- Glycogenolysis
- Lipolysis
- ketogenesis

↓

Enzymes of these pathways are inactive in dephosphorylated state

Note:

- Pathway not directly affected by insulin but linked to gluconeogenesis (affected by insulin) → Urea cycle
- Gluconeogenesis by urea cycle are interlinked as their substrates come from same molecule i.e. amino acids
- If one pathway upregulates the other also upregulates and vice – versa


Amino acids
Carbon skeleton
Nitrogen part
Substrate for gluconeogenesis
Metabolized by urea cycle

Questions:

1. Fate of enzymes in fasting or fed state?
2. Which enzymes are activated when insulin/Glucagon ratio ↑ or enzymes are inactivated when insulin/glucagon ration ↓?
3. Which enzymes are active in phosphorylated or dephosphorylated form?
4. Which pathways are upregulated or downregulated by insulin?

CHECKLIST FOR METABOLIC PATHWAY

- Tissue / organ
- Compartment
- Starting substrate / Donors
- End products
- Most important rate limiting enzyme
- Enzymes which are targeted for regulation
- Energetics

Reactions of Metabolic pathway

- Glycolysis
- TCA / Kreb's cycle
- Urea cycle

CARBOHYDRATES CLASSIFICATION – I

Carbohydrates: (CHO) 00:01:13

- Hydrates of Carbon $C_n (H_2O)_n$
- Molecular containing aldehyde or ketone derivatives of polyhydroxy alcohol
- Polyhydroxy aldehydes or Polyhydroxy ketones or their derivatives
- 55% to 60% of energy in diet is obtained from carbohydrates

Classification: 00:03:43

Carbohydrates

Depending on number of functional groups

Monosaccharides

- One functional group
- All monosaccharides are reducing sugars

Oligosaccharides

- 2 to 10 functional group
- Reducing / non – reducing sugars

Polysaccharides

- >10 functional groups
- All polysaccharides are Non – reducing sugars

Monosaccharides 00:05:53

- Depending on function group:

Monosaccharides

Aldoses (Aldehyde functional group)

Ketoses (Keto Functional group)

- Hydrolysis of monosaccharides do not produce two simple carbohydrates
- Depending on number of carbon atoms
  - Triose (C_3)
  - Tetrose (C_4)
  - Pentose (C_5)
  - Hexose (C_6)
  - Heptose (C_7)

Example	
Aldoses	**Ketoses**
Glyceraldehyde	Dihydroxyacetone
Erythrose	Erythrulose
Ribose	Ribulose
Glucose	Fructose
Glucose	Sedoheptulose

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Note:

- Simplest aldose → Glyceraldehyde

- Simplest Ketose → Dihydroxyacetone
- Glucose → Aldo hexose

Oligosaccharides 00:11:49

- 2 to 10 or 9 functional groups
- Depending on number of functional groups → Oligosaccharides
  - Disaccharides → Specific enzyme for their metabolism
  - Trisaccharide's and so an} Not completely digested → Reach large intestine in partially digested from → act as fuel for gut bacteria

Disaccharides

Reducing (Positive result for Benedict's test)	Non-reducing (Negative result for Benedict's test)
- Lactose, Lactulose	- Sucrose
- Maltose, Isomaltose	- Trehalose

- Note – Osazone Crystal test differentiates Lactose and maltose

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Lactose:

- Galactose + Glucose

  ↓ ↓

- Functional at group C1 Function Group C1
- B – Conformation
- Bond between galactose and glucose β – 1, 4 – Glycosidic bond
- Functional group of galactose at C1 is used in bond formation and Functional group of glucose at C1 is free → Reacts with benedict's reagent → Positive result → Hence reducing sugar
- Also known as milk sugar, amount in milk is 2% to 8% by weight
- Lactase (enzyme for digestion) – present in brush border epithelium of intestine at luminal side
- GI upset (infection / inflammation) → GI liming is shed off → Lactose also lost → Lactose cannot b metabolised → Hence milk and its products are avoided
- Lactose intolerance – Inherited deficiency of lactose

Physiology:

If lactose consumed

↓

Lactose not metabolism

↓

Reach Large intestine

↓

Digested by gut bacteria

↓

Sign and symptoms of GI system
[bloating, Flatulence, abdominal cramp,
Diarrhea, Vomiting (Extreme cases)]

Biochemical Finding

- Reducing substance in stool

Question

Which Milk product is safe in lactose intolerance?

A) Curd / Yogurt (Because bacteria in them metabolism lactose) Ice – cream (because of very less content of milk products and during the process of preparation lactose is broken down) Classification butter/Ghee

Maltose:

- Obtained from hydrolysis of starch
- Glucose + Glucose

  ↓ ↓

  Functional at group C1 Function Group C1
- Bond between two glucose molecules is Alpha – 1, 4 – Glycosidic bond
- Functional group of one glucose molecule at C1 is used in bond formation and functional group of

other glucose molecule at C1 is free → reacts with Benedict's reagent → Hence reducing sugar

- Also known as malt sugar
- Maltase (enzyme for digestion)

Sucrose

- Table sugar, source: Sugar cane / Sugar beet
- Glucose + Fructose

Glucose ↓ Functional at group C1

Fructose ↓ Function Group C1

- Bond Between glucose and fructose is Alpha – 1, 2 – Glycosidic bond
- No free functional groups → non – reducing sugar
- Sucrose (enzyme for digestion)
- Main transport from of carbohydrate in plants

DOT TUTORIALS LOGIN FOR SUCCESS

Trehalose

- Glucose + Glucose

Glucose ↓ Functional at group C1

Glucose ↓ Function Group C1

- Bond between two glucose molecules is Alpha – 1, 1 – Glycosidic bond
- Non-reducing sugar (as both the functional groups are used in bond formation)

CARBOHYDRATES CLASSIFICATION – II

Polysaccharides 00:00:23

- > 10 units (Units = Functional groups or number of monosaccharide units)

Polysaccharides

Depending on composition

Homopolysaccharides
- Subunits making the polysaccharide are all same

Heteropolysaccharides
- Subunits making the polysaccharide are not same

Homopolysaccharides

Depending on type of subunit

Glucose
↓
Glucan/Glucosan
Ex:
Starch
Glycogen
Cellulose
Dextrin
Dextran

Fructose
↓
Fructan/Fructosan
Ex: Insulin

N-Acetyl Glucosamine
↓
Chitin (Seen in insects)

- Note: Agar is not a homopolysaccharide it is a heteropolysaccharide (Subunit is modified galactose)

Starch

- Storage form in plants
- 2 types

  i. Amylose – Linear → $\alpha - 1, 4$ Glycosidic bond

  ii. Amylopectin – Branched

  - $\alpha - 1, 4$ – Glycosidic bond (Linear part)
  - $\alpha - 1, 6$ – Glycosidic bond (At branching)
- Branching appears after 24-26 residues → Known as poorly branched
- Note: Always amylose and amylopectin are obtained in a mixture from plants

Glycogen:

- Also known as animal starch (Storage form in animals)
- Highly branched (Branched after 10-18 residues average is after 12 residues)
- Stored only in liver, muscle, CNS

Cellulose

- Made of $\beta - 1,4$ Glycosidic bond (Can be broken by bacterial enzymes)
- Structural role (Strength to cell wall)

Dextrin:

- Obtained by hydrolysis of starch and glycogen

Dextran:

- Obtained from bacteria (Bacterial polysaccharide)
- Linear part: $\alpha - 1, 6$ – Glycosidic bond
- Branching site: $\alpha - 1, 3$ – Glycosidic bond
- Enzyme for its breakdown do not exist in human
- Used as plasma expander

Insulin

- Source: Tubers/Roots of dahlia family (Example – sunflower)
- Use: Storage form

Eligibility criteria for insulin in calculating renal clearance:

- Not digested
- Not absorbed
- Not metabolized
- Freely filtered
- Not reabsorbed
- Not secreted

Note:

- Insulin is not used clinically to calculate renal clearance because it has to be injected

- Creatinine, Cystatin C, Urea clearance are used clinically

Heteropolysaccharides: 00:14:49

- Hemicellulose
- Lignin
- Pectin
- Gum
- Glycosaminoglycans (GAGs)

GAGs: 00:16:25

- Repeating disaccharide having amino sugar and uronic acid (Amino sugar + uronic acid)
- Heavily negatively charged (Due to attached groups like phosphate, sulphate, acetyl)
  - Gel like nature
  - Acts as lubricant

Gags	Sites
Hyaluronic acid	Synovial fluid, vitreous humor, loose connective tissue
Chondroitin sulphate	Cartilage, bone, cornea
Keratan sulphate	Cornea (Type I) & Loose connective tissue (Type II)
Heparin	Mast cells
Heparan sulphate	Aortic wall skin fibroblasts
Dermatan sulphate	Wide distribution

- Gags + protein → proteoglycan/mucopolysaccharide
- Mucopolysaccharide depending on complex → i. Mucopoluysaccharide monomer ii. Mucopolysaccharide aggrecan

Mucopolysaccharidosis: 00:23:53

- Defective degradation of mucopolysaccharide – Due to inherited genetic defect in which there is deficiency of one or more enzymes

Clinical features of mucopolysaccharidosis (MPS):

- Musculo skeletal deformities
- Poor growth
- Cardiac abnormalities
- Mental retardation
- Corneal scarring

MPS type	Name of the syndrome	Enzyme defect
MPS I	Hurler's disease (Severe) H-S type Scheie disease (Mild)	Iduronidase
MPS II	Hunter's disease	Iduronate sulfatase
MPS III	Sanfilippo (Subtypes A, B, C, D)	

MPS IV	Maroteaux disease (Subtypes A, B)	
MPS VI	Maroteaux – Lamy disease	Aryl sulfatase – B
MPS VII	Sly disease	β - Glucuronidase

Note:

- MPS V – Reclassified as MPS I
- Enzyme replacement therapy is available for MPS I, II and VI
- Inheritance of most in born errors of metabolism is autosomal recessive, but MPS type II is X-linked recessive inheritance
- MPS type II – no corneal scarring
- Scheie disease (MPS I S) – Minimal metal retardation

INDENTIFICATION OF CARBOHYDREATES

Test for identification of carbohydrates: 00:00:59

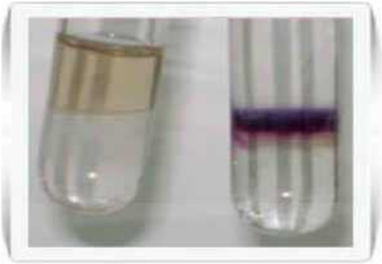

Fig: 1

I. Molisch Test:

- Concentrated H_2SO_4 (sulphuric acid) + any carbohydrate

$$\downarrow \text{Oxidation}$$

Furfural compounds at junction

$$\downarrow$$

Purple ring with brown base

Molisch Vs Rothera's test

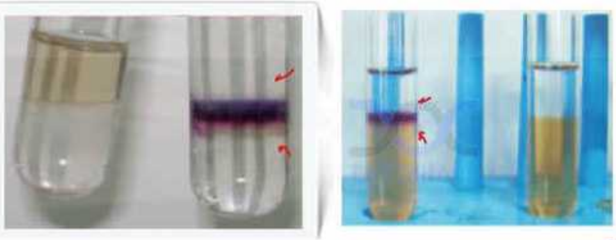

Fig: 2

- Molisch's –upper and lower portion, i.e. above and below the ring are clear
- Rothera's – upper layers is clear

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  - Lower layer is dirty brown (due to saturated salt segment)
- Not: Rothera's test: test for ketone bodies, also known as modified nitroprusside test

Iodine Test 00:03:53

- Used for polysaccharides (starch & glycogen)
- Not a chemical reaction, it is physical adsorption phenomenon
- Starch → blue black color
- Glycogen → red / wine color
- On heating a positive iodine test, the color disappears because iodine molecules attain extra kinetic energy, so they move away from the surface of carbohydrates

Benedict's test 00:06:09

- For reducing substance like
  - Reducing sugars
  - Uric acid
  - Creatinine
  - Ascorbic acid
- Benedict's reagent – contains oxidised copper
- Semi – Quantitative test – as it gives an idea of approximate concentration of sugar
- Benedict's test
  - Positive for all monosaccharides

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- Mixed picture for disaccharides (some are positive, some are negative)
- Negative for polysaccharides (as they don't have sufficient functional groups per unit mass to give positive result)

- Benedict's test
  - Positive – for reducing sugar
  - Negative – for non-reducing sugar

Colors and corresponding concentration 00:09:23

Solution / precipitate	Glucose concentration
Benedict's solution (blue)	Nil
Green solution	0 to 0.5 mg%
Green precipitate	0.5 to 1.0mg%
Yellow precipitate	1.0 to 1.5 mg%
Orange Precipitate	1.5 to 2.0 mg %
Brick red precipitate	>2.0 mg%

Note:

- Difference between solution and precipitate → when paper placed behind the tube

Able to visualize or read the paper
↓
Solution

Unable to visualize or read the paper
↓
Precipitate

- Mnemonic for colors of Benedict's test: like the rainbow: VIBGYOR
- V1
  - B – Blue
  - G_2 – Green
  - Y – Yellow
  - O – Orange
  - R – Red

- The precipitate in Benedict's test that settles at the bottom of the tube, irrespective of the glucose concentration is always brick red because as cuprous oxide is formed in all tubes (cuprous oxide = brick red color)
- The reason for different colors (green, yellow, orange, red) is that the formed cuprous oxide gets suspended in the mixture and it's interaction with the background blue color of benedict's reagent

Basford's test 00:14:14

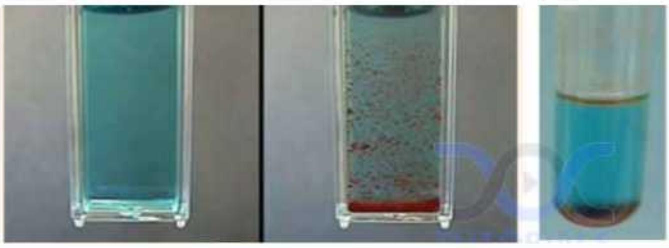


Fig: 3

- Differentiate reducing monosaccharide and disaccharides

- Positive for reducing monosaccharide as they have higher free functional groups per unit mass to react with copper

Seliwanoff's test 00:14:45

Fig: 4

- Positive for keto group (fructose)
- Cherry red color (positive test) – indicates presence of "ketose"→ sugar with keto functional group

Osazone Crystals 00:15:37

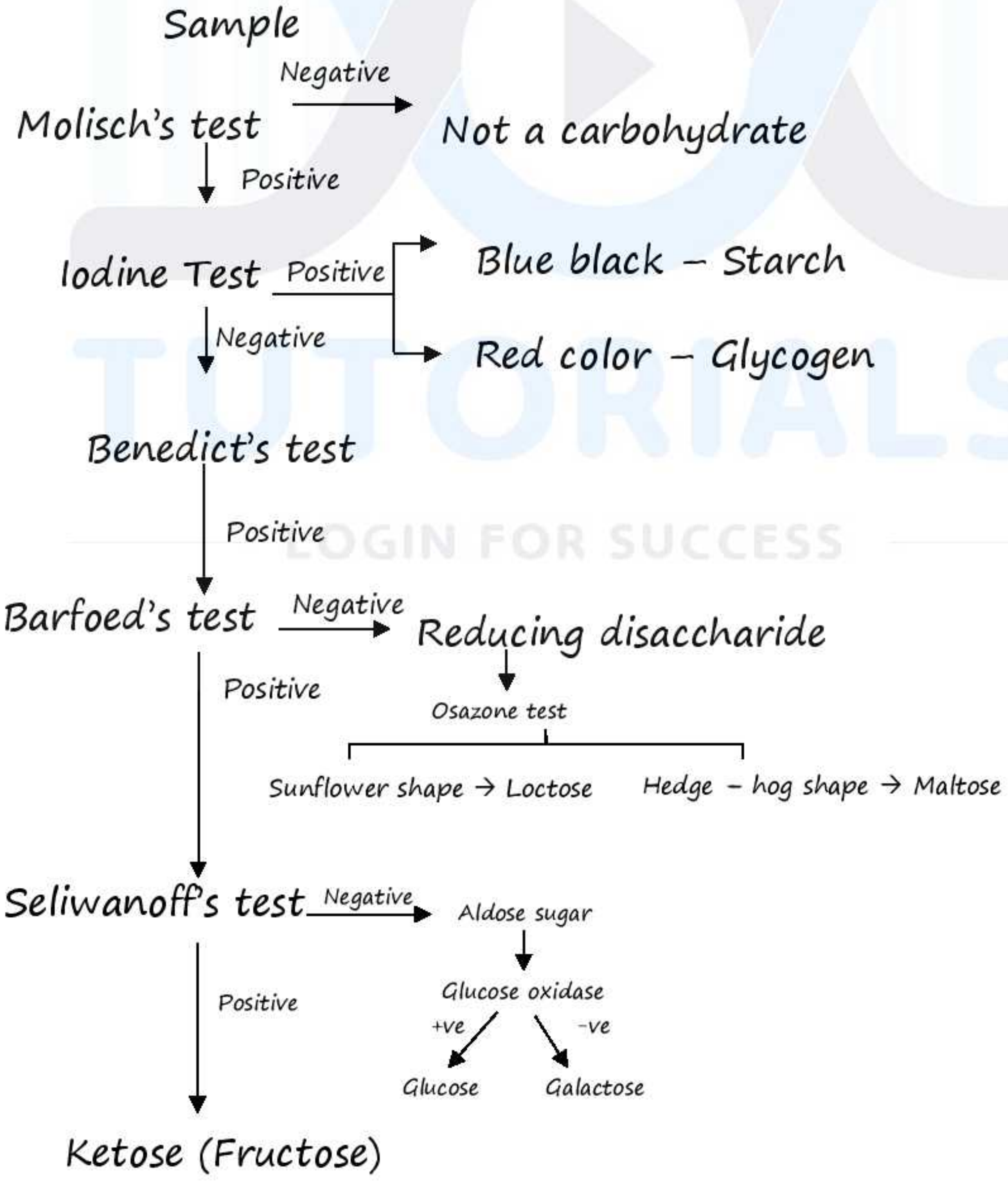
- To differentiate reducing disaccharides

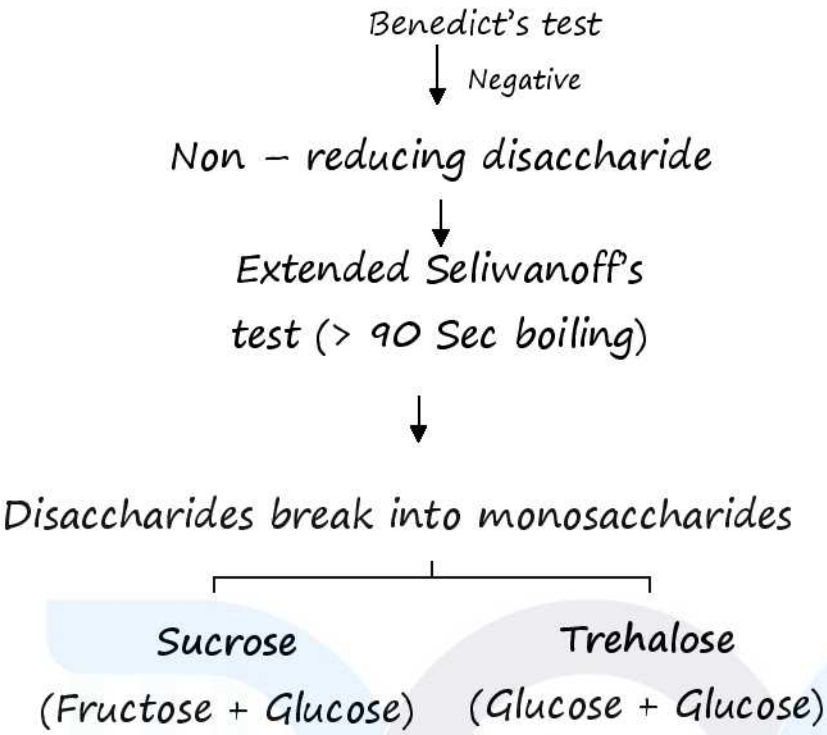
Sugar	Osazone crystal
Maltose	Sunflower shaped
Lactose	Pin cushion / hedgehog / cotton ball shaped
Galactose	Envelope / rhomboid shaped
Glucose / Fructose / Mannose	Needle shaped

- Note: Osazone crystal formation involves first 2 carbon atoms so the difference in the first 2 carbon atoms of glucose, fructose and rest of the molecules becomes similar, giving similar needle shaped crystals
- Glucose and Mannose – epimerization at C_2
- Glucose – Functional group at C_1
- Fructose – Functional group at C_2

Flowchart for identification of carbohydrate

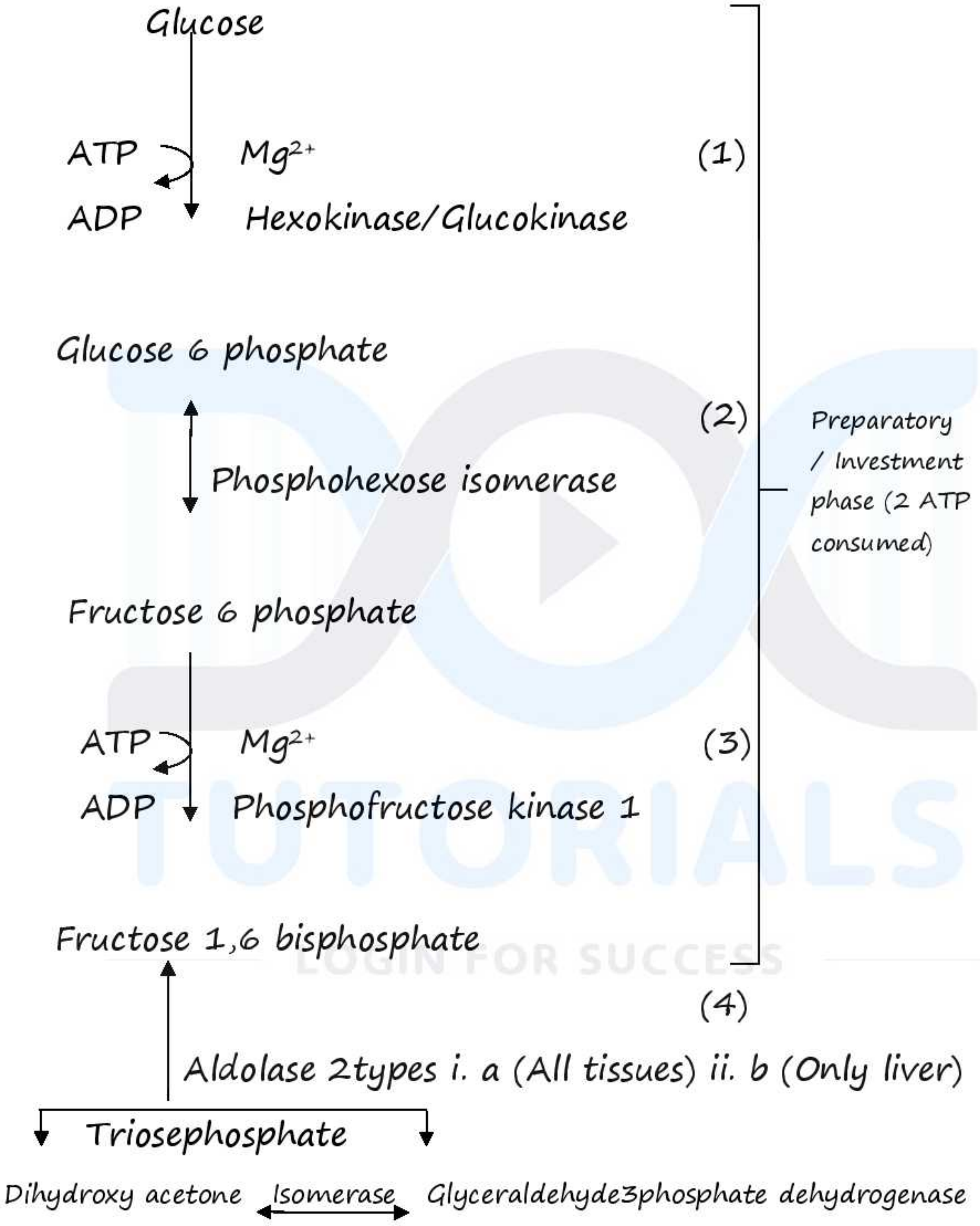
00:18:37


Sample
Negative
Molisch's test
Not a carbohydrate
Positive
Iodine Test
Positive
Blue black – Starch
Negative
Red color – Glycogen
Benedict's test
Positive
Barfoed's test
Negative
Reducing disaccharide
Positive
Osazone test
Sunflower shape → Loctose
Hedge – hog shape → Maltose
Seliwanoff's test
Negative
Aldose sugar
Glucose oxidase
+ve
-ve
Glucose
Galactose
Positive
Ketose (Fructose)


Benedict's test
Negative
Non – reducing disaccharide
Extended Seliwanoff's test (> 90 Sec boiling)
Disaccharides break into monosaccharides
Sucrose
(Fructose + Glucose)
Trehalose
(Glucose + Glucose)

GLYCOLYSIS - REACTIONS

Glycolysis 00:00:50


Glucose
ATP
ADP
Mg2+
Hexokinase/Glucokinase
(1)
Glucose 6 phosphate
Phosphohexose isomerase
(2)
Fructose 6 phosphate
ATP
ADP
Mg2+
Phosphofructose kinase 1
(3)
Fructose 1,6 bisphosphate
(4)
Aldolase 2types i. a (All tissues) ii. b (Only liver)
Triosephosphate
Dihydroxy acetone
Isomerase
Glyceraldehyde3phosphate dehydrogenase
Preparatory / Investment phase (2 ATP consumed)

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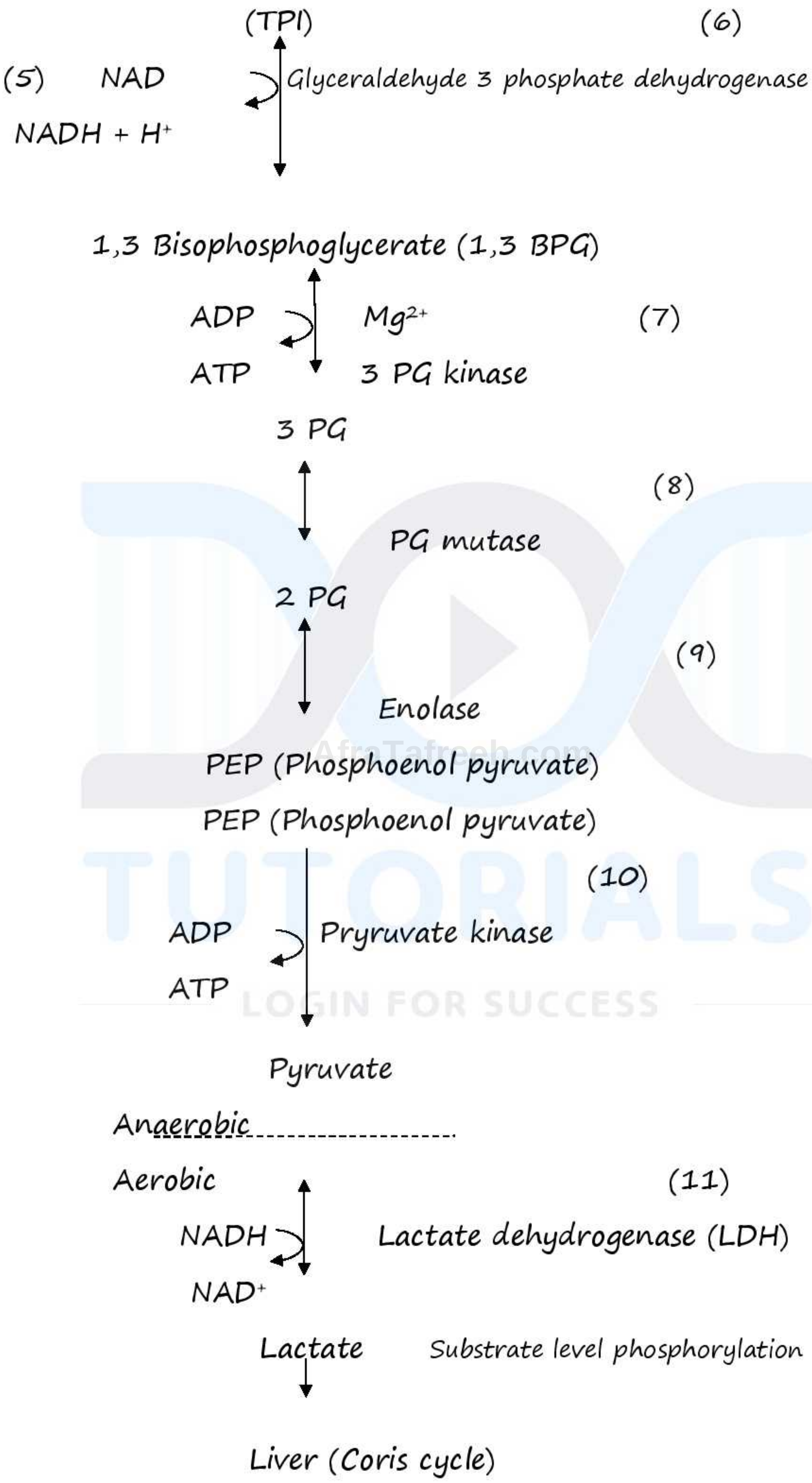
(TPI)
(6)
(5)
NAD
NADH + H+
Glyceraldehyde 3 phosphate dehydrogenase
1,3 Bisophosphoglycerate (1,3 BPG)
ADP
ATP
Mg2+
3 PG kinase
(7)
3 PG
(8)
PG mutase
2 PG
(9)
Enolase
PEP (Phosphoenol pyruvate)
PEP (Phosphoenol pyruvate)
(10)
ADP
ATP
Pryruvate kinase
Pyruvate
Anaerobic
Aerobic
(11)
NADH
NAD+
Lactate dehydrogenase (LDH)
Lactate
Substrate level phosphorylation
Liver (Coris cycle)

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- Reactions of glyceraldehyde 3 phosphate occurs twice as DHAP also converts into glyceraldehyde 3 phosphate
- In aerobic glycolysis → NADH enters ETC → NAD$^+$ Liberated
- In anaerobic glycolysis →

NADH does not enter ETC → no NAD$^+$ replenishment → (-)

Regenerates NAD$^+$ → (+) Glycolysis

$$\text{Pyruvate} \longrightarrow \text{Lactate}$$

- 3 irreversible reactions of glycolysis catalysed by

i. Hexokinase/Glucokinase → Reaction 1

ii. PFK – 1 → Reaction 3

iii. Pyruvate kinase → Reaction 10

- $$\text{Glycogen} \xrightarrow{\text{Glycogen phosphorylase}} \text{Glucose 1 phosphate} \leftrightarrow \text{Glucose 6 phosphate}$$

↑

Pi

↑

Pyridoxal phosphate

- So, ATP is not consumed hence net gain of ATP is 3 when glycogen is fuel

- In RBC – Rapaport leubering shunt

(1st substrate level phosphorylation by passed for some of trioses)

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1,3 BPG

↓

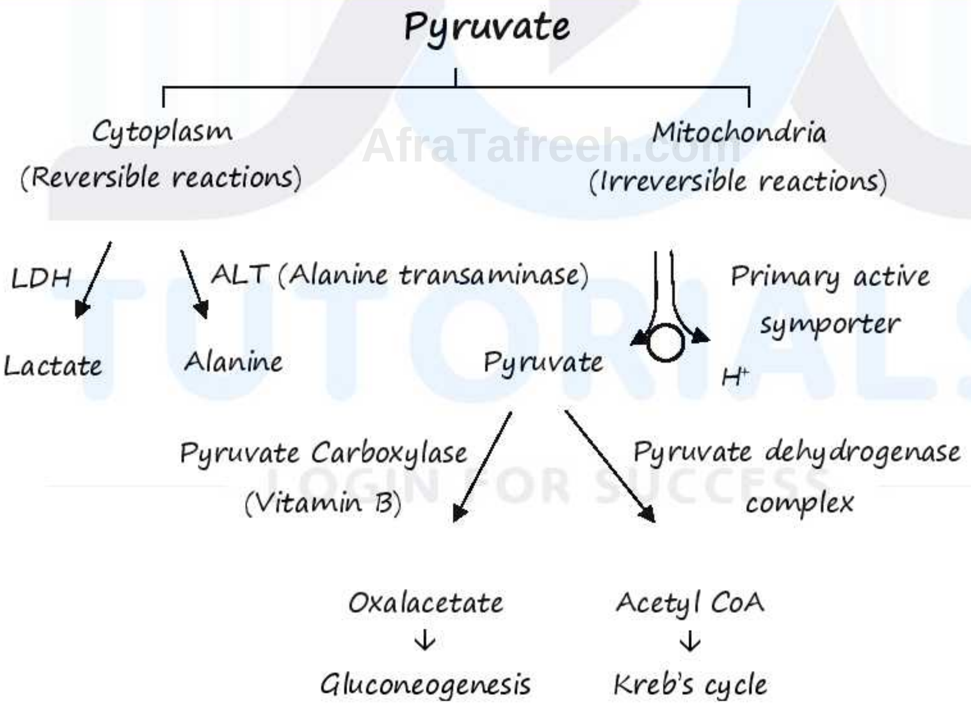
2,3 BPG (Shifts hemoglobin-oxygen dissociation curve to right)

↓

3 PG

- At high attitude – More 2,3 BPG required → All trioses form 2,3 BPG → 0 ATP
- At sea level – Less 2,3 BPG required → Only one triose to 2,3 BPG → 1 ATP

Fate of pyruvate 00:23:29


Pyruvate
Cytoplasm
(Reversible reactions)
Mitochondria
(Irreversible reactions)
LDH
Lactate
ALT (Alanine transaminase)
Alanine
Primary active
symporter
H⁺
Pyruvate
Pyruvate Carboxylase
(Vitamin B)
Pyruvate dehydrogenase
complex
Oxalacetate
Gluconeogenesis
Acetyl CoA
Kreb's cycle

Note:

- Primary active symporter
- Transfer pyruvate and proton (H^+) inside the mitochondria
- Requires ½ ATP per pyruvate
- Link reaction

$$\text{Pyruvate} \xrightarrow{\text{Pyruvate dehydrogenase complex}} \text{Acetyl CoA}$$

GLYCOLYSIS HIGHLIGHTS

Glycolysis 00:01:03

- 1st pathway for hexose metabolism (Glucose, Galactose, Fructose)
- Also known as EMP (Embden-Meyerhof-Parnas) Pathway
- 2 Phases
  i. Preparatory/Investment phase
  ii. Pay off phase
- Depending on availability of oxygen
  i. Aerobic glycolysis
  ii. Anaerobic glycolysis
- Location:
- Exclusively anaerobic
  i. RBC – because of no mitochondria
  ii. Cornea
  iii. Lens
  iv. Sub-epithelial layer of skin and GIT

  Because avascular

Note:

- Outermost layer of cornea gets oxygen from atmosphere through tear film, hence aerobic glycolysis occurs
- Mixed glycolysis (Anaerobic + aerobic glycolysis even in resting state):

i. CNS (Energy requirement of CNS is 40% of total energy produced)

Aerobic glycolysis (From 20% oxygen supplied by blood) | Anaerobic glycolysis

ii. Renal medulla (Because of vasa recta containing venous blood i.e., low oxygen)

- Exclusively aerobic at resting state: All others
- Compartment: Cytoplasm
- Substrates: Glucose, Galactose, Fructose, Glycogen (Not a direct substrate)

End products

Anaerobic ↓	Aerobic ↓
- 1 mole of glucose (+) 2 moles of NAD$^+$	- 1 mole of glucose (+) 2 mole of NAD$^+$
- 2 moles of lactate (+) 2 moles of NAD$^+$	- 2 moles of pyruvate (+) 2 moles of NADH + H$^+$

- Rate limiting enzyme (Most important)
- Phosphofructokinase – 1 (PFK–1) > Hexokinase / Glucokinase

Physiological regulation 00:13:20

i. Hexokinase

- Feedback inhibition by glucose – 6 – phosphate

- Not affected by hormonal changes

ii. PFK – 2

- Hormonal regulation by phosphorylation and dephosphorylation (insulin, glucagon, epinephrine, growth hormone are involved)

iii. PFK – 1

Allosteric regulation

Activators	Inhibitors
- Fructose 6 phosphate	- ATP
- Fructose 2,6 Bisphosphate	- Citrate/Citric acid in cytoplasm
- AMP	

Note:

- Feed forward activation: Fructose 6 phosphate – Substrate for PFK – 1; substrate is activating it' s own enzyme
- Fructose 2,6 Bisphosphate – Product of action of PFK – 2
- Fructose 6 phosphate → PFK – 2 → fructose 2,6 bisphosphate
- AMP – Describes the energy state of cell
- High AMP - Cell has low energy → Glycolysis activated
- Low AMP – Cell has sufficient energy → Glycolysis not required

iv. Glucokinase

- Activity increased by insulin
- Enzyme concertation increases (i.e. insulin promotes synthesis of fresh enzyme) but not individual activation of enzyme

v. Pyruvate kinase

- Also activated by insulin

External/Non-physiological regulation 00:19:50

---

i. Enolase – Inhibited by fluoride (NAF)

- Sodium fluoride (NaF) is a weak anticoagulant, hence always used with a strong anticoagulant

ii. Glyceraldehyde – 3 – PO_4 dehydrogenase – Inhibited by iodoacetate

iii. 3 – Phosphoglycerate kinase – Affected by arsenite

- Arsenite – Doesn't inhibit glycolysis
- By passes the action of 3 – Phosphoglycerate kinase, thereby no ATP synthesis, hence arsenite also known as uncoupler of glycolysis
- 1,3 – Bisphosphoglycerate

  ↓

  Intermediate by arsenite –

  ↓
- 3 – Phosphoglycerate

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Energetics 00:23:40

Allosteric regulation

- Anaerobic
  - In RBC

    ↓

    Rapaport leubering shunt

    ↓

    0 or 1 ATP (Depending on altitude)
  - In most cells:

    ↓

    2 ATP
  - In muscles (Glycogen as fuel)

    ↓

    3 ATP
- Aerobic
  - CNS, skeletal muscles

    ↓

    Glycerol-3-phosphate shuttle

    ↓

    5’ ATP
  - Liver, kidney, heart

    ↓

    Malate-Aspartate shuttle

    ↓

    7 ATP

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GLUCONEOGENESIS

Introduction 00:00:43

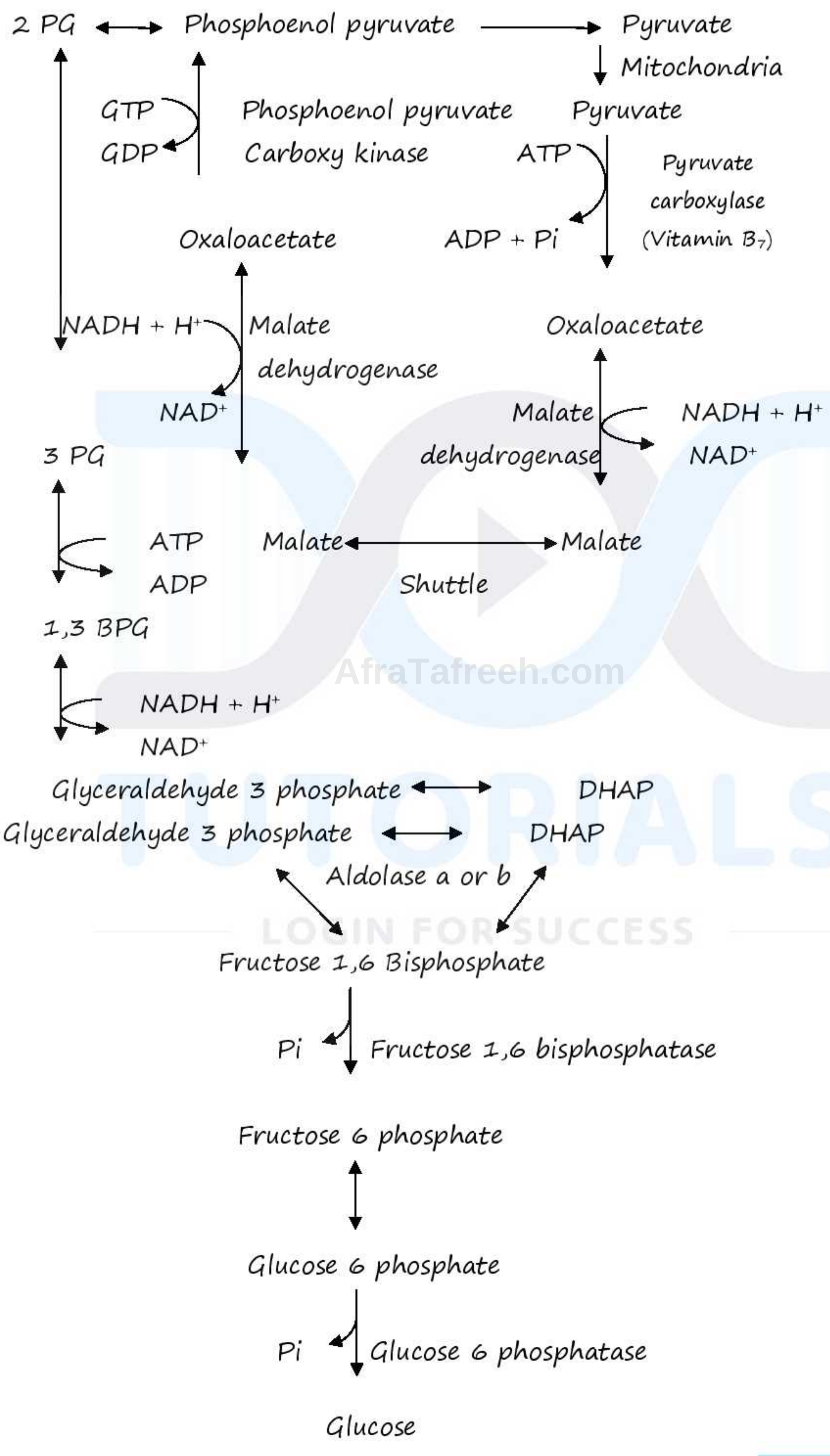
- Compartment: Mitochondria and cytoplasm
- Location: Liver and kidney (When defective, intestine may be involved)
- Substrates (Non carbohydrate sources)
  - i. Glucogenic amino acids
    - All amino acids are glucogenic except lysine and leucine (Ketogenic)
    - Alanine – Most important contributor
    - Alanine ⟷ glucose (Cahill cycle)
  - ii. Lactate
  - iii. Pyruvate
  - iv. Glycerol
  - v. Odd chain fatty acid (Last 3C → Propionyl CoA)

Note:

- All the substrates directed towards gluconeogenesis via oxaloacetate mostly
- Rate limiting enzyme ┐ Fructose 1, 6 Bisphosphatase
- Target for regulation ┘

TUTORIALS LOGIN FOR SUCCESS

Pathway 00:06:30

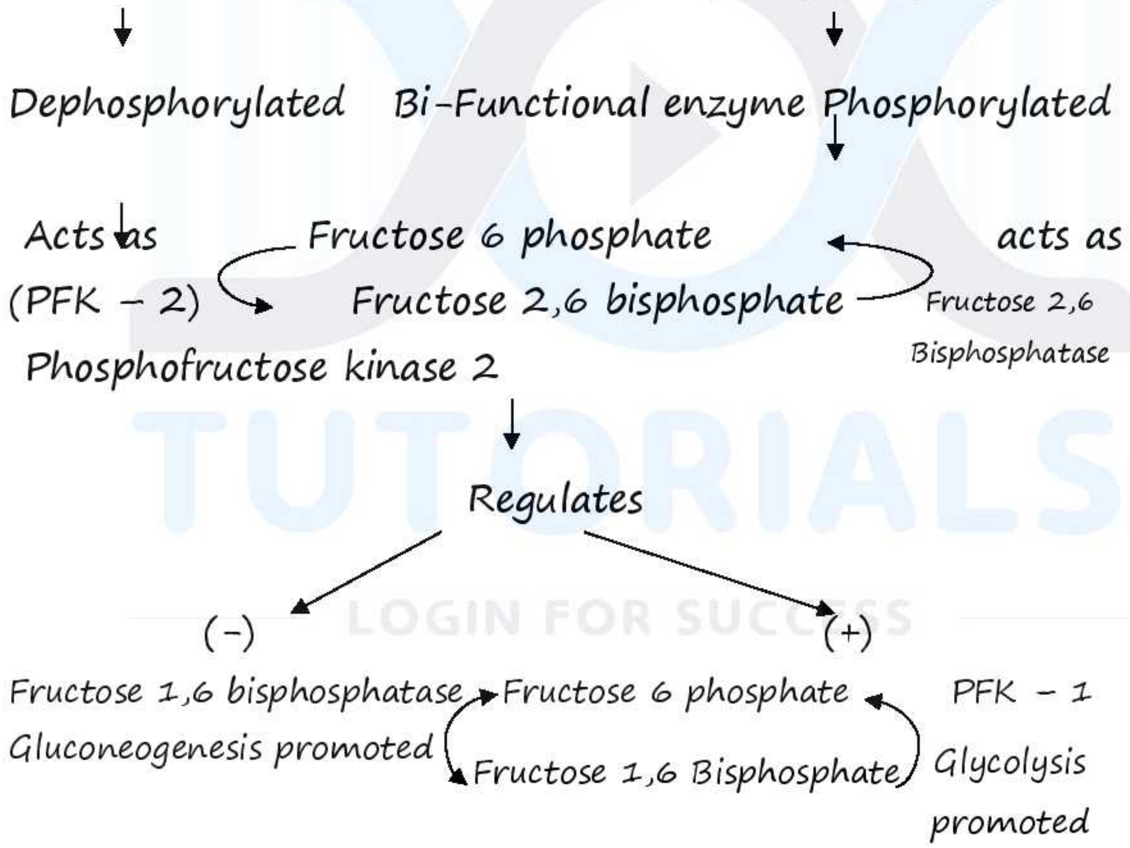

2 PG
Phosphoenol pyruvate
Pyruvate
Mitochondria
GTP
GDP
Phosphoenol pyruvate
Carboxy kinase
Pyruvate
ATP
Pyruvate
carboxylase
(Vitamin B_7)
Oxaloacetate
ADP + Pi
NADH + H^+
Malate
dehydrogenase
Oxaloacetate
NAD^+
Malate
dehydrogenase
NADH + H^+
NAD^+
3 PG
ATP
Malate
Malate
ADP
Shuttle
1,3 BPG
NADH + H^+
NAD^+
Glyceraldehyde 3 phosphate
DHAP
Glyceraldehyde 3 phosphate
DHAP
Aldolase a or b
Fructose 1,6 Bisphosphate
Pi
Fructose 1,6 bisphosphatase
Fructose 6 phosphate
Glucose 6 phosphate
Pi
Glucose 6 phosphatase
Glucose

Note:

- Target for regulation: Fructose 1,6 Bisphosphatase
- Most potent stimulator: Glucocorticoid (Cortisol) – Produced in response to stress
- Glucagon and epinephrine – Other stimulators
- Insulin – Inhibitor

Counter Regulation between glycolysis and gluconeogenesis: 00:18:08

- It is maintained by a bi-functional enzyme
- Insulin Glucortiocoids, glucagon, epinephrine


Dephosphorylated
Bi-Functional enzyme
Phosphorylated
Acts as
(PFK – 2)
Phosphofructose kinase 2
Fructose 6 phosphate
Fructose 2,6 bisphosphate
acts as
Fructose 2,6
Bisphosphatase
Regulates
(–)
(+)
Fructose 1,6 bisphosphatase
Gluconeogenesis promoted
Fructose 6 phosphate
Fructose 1,6 Bisphosphate
PFK – 1
Glycolysis
promoted

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Energetics: 00:22:47

- 2 moles of pyruvate → 1 mole of glucose
- 2 ATP → Used in mitochondria
- 2 GTP
- 2 ATP
- 2 NADH + H^+

(2 GTP, 2 ATP, 2 NADH + H^+) Used in cytoplasm

~ 11 ATP equivalents used

- Gluconeogenesis is ~ 35 to 40% costlier in comparison to glycolysis (1 mole of glucose → 2 mole of pyruvate → 7 ATP gain)

HMP SHUNT

HMP (Hexose Mono Phosphate) Shunt: 00:00:34

- Also known as PPP (Pentose Phosphate Pathway) → Due to Ribose – 5 – Phosphate formation
- Alternate pathway for glucose metabolism in cytoplasm
- When glycolysis is saturated → HMP shunt
- 2 phase
  - Oxidative phase
    - i. NADPH + H^+ synthesized
    - ii. All reaction are irreversible
  - Non – Oxidative phase – Ribose 5 Phosphate production → purine and pyrimidine synthesis (Nucleic acid synthesis)
  - Reversible reaction
- Compartment – Cytoplasm

Role of NADPH + H^+ 00:03:59

- (Reducing equivalents for Biosynthesis and Antioxidant)
- Provide reducing equivalent for fatty acid biosynthesis
- Steroid Biosynthesis
- Cholesterol biosynthesis
- Reduce glutathione (anti – oxidant)
- Drug metabolism

- Detoxification
- Required for oxidative burst in WBC
- RBC membrane stabilization

Tissue in which HMP Shunt is upregulated

00:06:19

- Testis (steroids hormone formation)
- Thyroid (thyroxine hormone synthesis)
- Adrenal cortex (hormone synthesis)
- Adipose tissue (fatty acid synthesis)
- Liver (cholesterol synthesis, drug metabolism and detoxification)
- Lactating breast (fatty acid synthesis for milk)
- RBC (for membrane stabilization)
- Lens of eye (antioxidant role)

Substrates and Products 00:08:12

- 3 molecules of glucose 6 phosphate + 6 molecule of $NADP^+$

↓

2½ molecules of glucose 6 phosphate

+

3 molecules of CO_2 + ½ Pi

+

6 molecules of NADPH + H^+

Oxidative Phase 00:10:15

Glucose 6

$NADP^+$

$NADPH + H^+$ ↓ Glucose 6 Phosphate dehydrogenase

6 Phosphogluconate (C_6)

$NADP^+$

$NADPH + H^+$ ↓ 6 – Phosphoglucomate dehydrogenase

Rinbulose 5 Phosphate (C_5)

Non – Oxidative Phase: 00:13:17

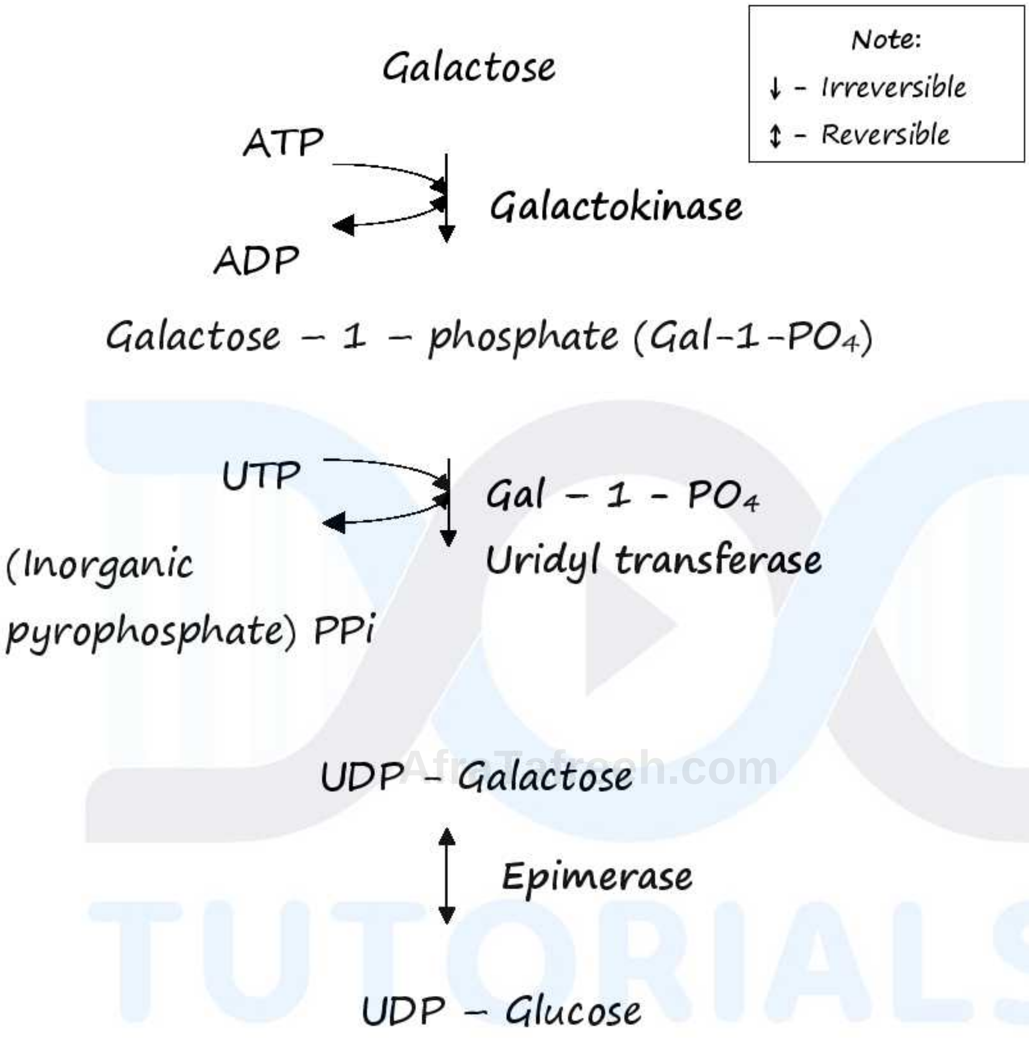
- Transketolase (RBC) → used to monitor deficiency of B_1

Note

- G6PD
  - Rate limiting enzyme
  - Target for regulation
  - Upregulated by insulin

GALACTOSE METABOLISM

Galactose metabolism pathway 00:00:40


Note:
↓ - Irreversible
↕ - Reversible
Galactose
ATP
ADP
Galactokinase
Galactose – 1 – phosphate (Gal–1–PO4)
UTP
(Inorganic
pyrophosphate) PPi
Gal – 1 – PO4
Uridyl transferase
UDP – Galactose
Epimerase
UDP – Glucose

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- UDP – Galactose: Used in synthesis of:
  - Glycoproteins
  - Glycolipids
  - Glycosaminoglycans (GAGs)
  - Lactose (lactating mammary glands)
  - Uronic acid → GAGs

Galactosemia 00:04:39

- Inborn error of metabolism of carbohydrates

- Due to deficiency of any of the above 3 enzymes
- Most common enzyme deficiency causing galactosemia is Galactose – 1 – phosphate uridyl transferase

Clinical features 00:05:15

- Presentation in 1st week of life (3-6 days) – because of the presence of galactose i.e., lactose (glucose + galactose) in breast milk
- Usually presents to emergency department
- Neurological signs and symptoms like vomiting, lethargy, decreased responsiveness, poor feeding
- Oil drop cataract – at birth or with in 1 year of age
  - Pathogenesis: Accumulation of galactose in lens

    ↓ Aldose reductase

    Galactitol in lens
  - It is usually reversible
- Urine examination
  - Benedicit's test positive (as galactose is a monosaccharide)
  - To differentiate glucose and galactose

↓

Glucose oxidase test

Positive — Negative

Glucose — Galactose

Management 00:08:02

- Dietary restriction of milk
- Galactose free top feeds

Note:

Epimerase

Present	**Deficient**
• UDP – Galactose which is used for various synthesis like glycoproteins, glycolipids, GAGs etc can be formed from UDP – Glucose • So complete diet restriction of galactose can be done	• No UDP – Galactose ↓ No synthesis of glycoproteins, glycolipids, GAGs etc., which are important for neurological development • So, some amount of galactose is allowed in the diet

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FRUCTOSE METABOLISM

Fructose absorption 00:00:57

Fructose in diet

↓

Uptake by facilitative transporter (GLUT-5)
(no active transport for fructose in intestine)

↓

- Incomplete absorption of fructose from diet because facilitative transport occurs along concentration gradient
- When excess fructose taken in one meal → causes osmotic diarrhea

↓

Because of more fructose left in GIT (Gastro-Intestinal Tract)

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Metabolism of fructose 00:03:31

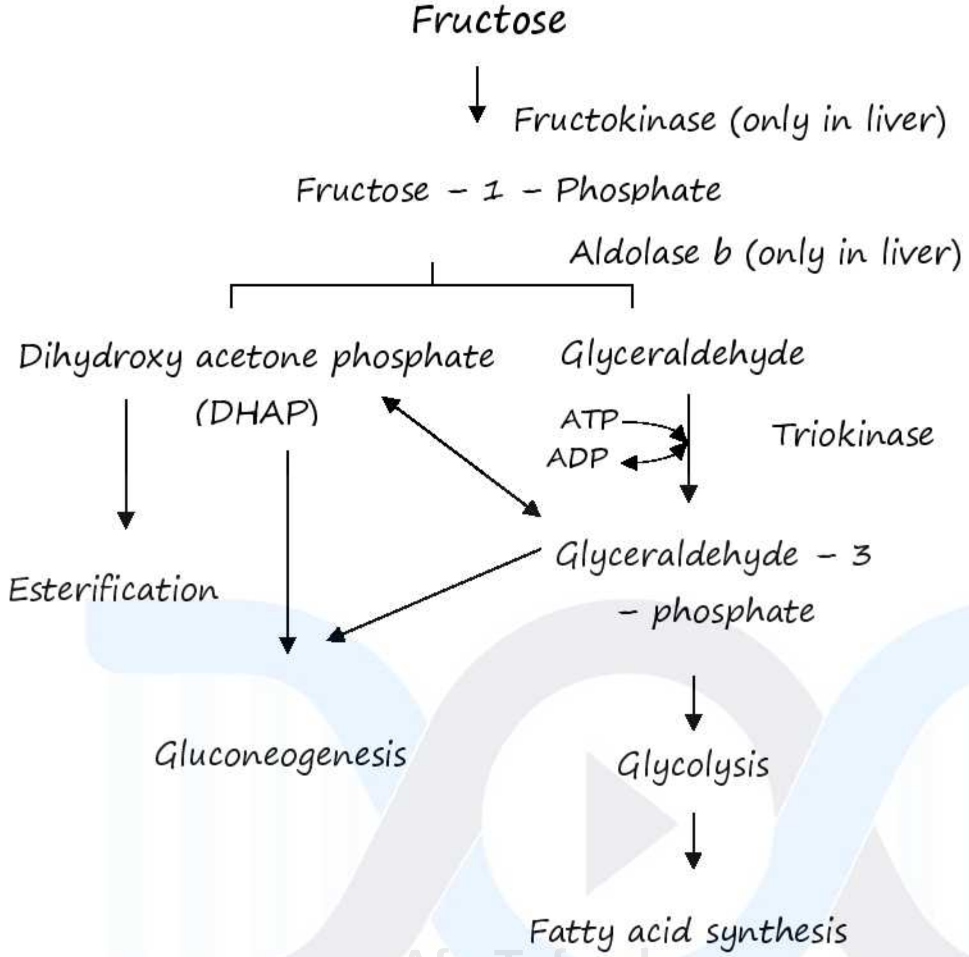
- In liver – rapidly metabolized (because of no regulatory steps)
- In extra-hepatic tissues – metabolism mimics glucose metabolism

Fructose

↓ **Hexokinase** (higher affinity or lower k_m for glucose than fructose)

Fructose-6-phosphate

In liver


Fructose
Fructokinase (only in liver)
Fructose – 1 – Phosphate
Aldolase b (only in liver)
Dihydroxy acetone phosphate (DHAP)
Glyceraldehyde
ATP
ADP
Triokinase
Glyceraldehyde – 3 – phosphate
Esterification
Gluconeogenesis
Glycolysis
Fatty acid synthesis

- Note: No regulation of fructokinase and aldolase-b
- If excess fructose consumed ─ acute response – fatty liver
  chronic response – obesity

↓

↑ Fatty acid synthesis

And

↑ Esterification

↓

↑ VLDL (Triacyl glycerol/ TAG)

↓

↑ LDL → Atherosclerosis

Normally in tissue 00:11:58

Glucose

↕ Aldose reductase

Sorbitol

↕ Sorbitol dehydrogenase (liver, seminal vesicles, lens)

Fructose

Note

- In diabetics → increase glucose

↓

Increase sorbitol and increase fructose

↓

Diabetic cataract

- Excess fructose in lens due to deficiency of fructokinase or aldolase-b causes cataract

Abnormal fructose metabolism 00:14:32

- Deficiency of fructokinase
- Deficiency of aldolase-b

Deficiency of fructokinase

- Known as essential fructosuria
- No signs and symptoms, incidental finding
- Identified at any age
- Urine examination
  - Positive Benedict's test
  - Negative glucose oxidase test

  - Confirmatory seliwanoff's test (positive)
- At 12-15 years of age – diabetes like cataract

Deficiency of aldolase-b

- Known as hereditary fructose intolerance

Fructose

↓

Fructose – 1 – phosphate → phosphate is trapped

Aldolase – b

inhibits glycogenolysis

decrease ATP synthesis

⊖ Decrease ATP synthesis

Feedback inhibition of purine synthesis – By end products of purine synthesis

→ Increase purine synthesis

→ Increase uric acid formation

→ Hyperuricemia

- In liver, phosphate is required for glycogenolysis

Phosphate is trapped

↓ ⊖

Glycogenolysis

↓

No Glucose – 1 – phosphate

↓

Profound hypoglycemia

↓

Coma, restless, decreased responsiveness vomiting

- Age of presentation: > 6 months

because

Exclusive breast feeding till 6 months (no fructose)

Weaning after 6 months (top feeds – fruits – contain fructose)

- Usually presents to emergency department with neurological signs and symptoms
- No cataract at time of presentation
- Urinary finding – same as essential fructosuria

GLYCOGEN METABOLISM

Glycogen

- Homopolysaccharide of glucose
- Storage form
- Highly branched
- Stored in liver, muscle, brain

liver → Maintains blood glucose level

muscle, brain → used for local energy needs

	% of weight	Total content
Liver glycogen	5% (weight of liver – 1.8kg)	90gm
Muscle glycogen	0.7% (weight of muscle – 35kg)	245gm

Glycogenin

- 37k Da protein
- Anchor for glycogen
- Provides glycogen primer (by enzymatic action)

Glycogen primer synthesis

Anchor function of glycogenin – Hydroxyl group of Tyrosine residue undergo glycosylation

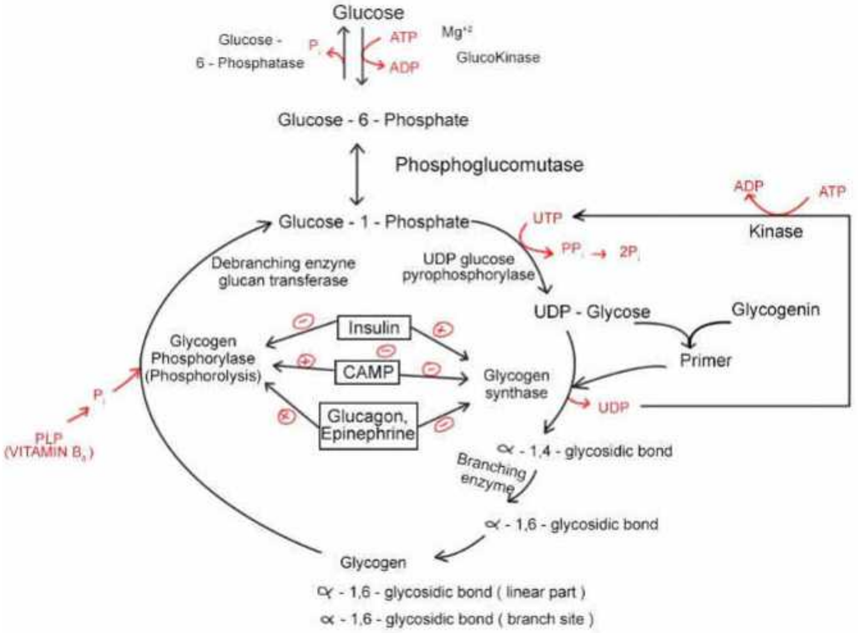
↓

Addition of 7 more glucose residues linked via α-1,4 – glycosidic bond

(By enzymatic action of glycogenin)

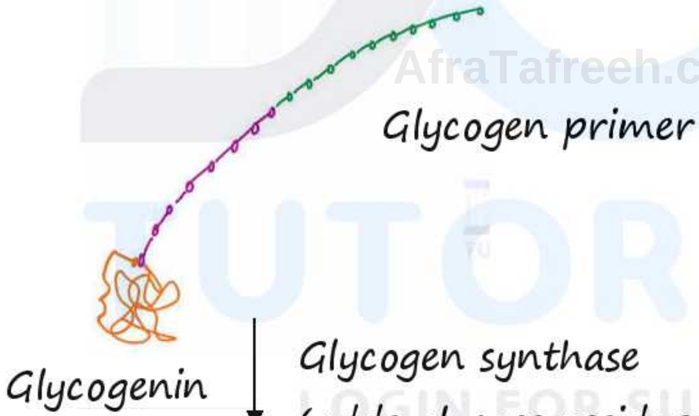
Note:

- In muscle, all glycogen is anchored to glycogenin (because relative concentration of glycogen is less)
- In liver, glycogen may or may not be anchored to glycogenin (because relative concentration of glycogen is high)
- Once glycogenin has synthesized a long glycogen, it will dissociate from glycogen and synthesize a new primer which take part in synthesis of another glycogen

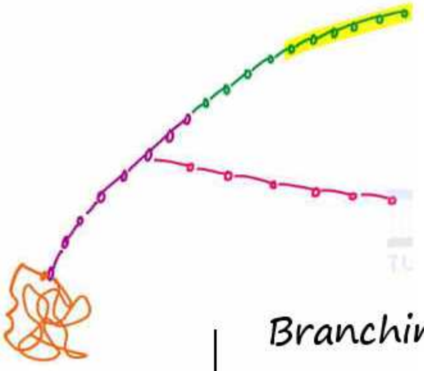

Glucose
Glucose - 6 - Phosphatase
P_i
ATP
ADP
Mg^{+2}
GlucoKinase
Glucose - 6 - Phosphate
Phosphoglucomutase
Glucose - 1 - Phosphate
UTP
PP_i → $2P_i$
ADP
ATP
Kinase
Debranching enzyne glucan transferase
UDP glucose pyrophosphorylase
UDP - Glycose
Glycogenin
Primer
Insulin
CAMP
Glucagon, Epinephrine
Glycogen Phosphorylase (Phosphorolysis)
Glycogen synthase
UDP
P_i
PLP (VITAMIN B_6)
∝ - 1,4 - glycosidic bond
Branching enzyme
∝ - 1,6 - glycosidic bond
Glycogen
∝ - 1,6 - glycosidic bond (linear part)
∝ - 1,6 - glycosidic bond (branch site)

- "UDP Glucose"
- α-1,4 glycosidic bond (linear part)
- Show release of free glucose at debranching enzyme
- When the branch site chain is small, 2 enzymes are required
  - Glucan Transferase – decrease the side chain as only branch residue
  - Debranching enzyme – cleaves the branch site by hydrolysis, thereby free glucose is released

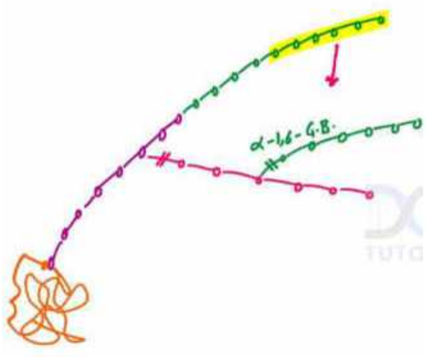
Glycogenesis



↓ Glycogen synthase (adds glucose residues)

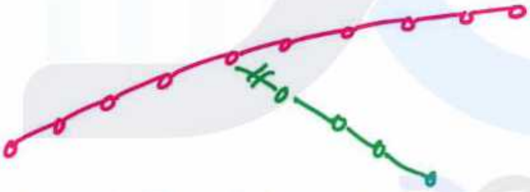


↓ Branching enzyme (transfers the last six residues to an adjoining chain once the length of the chain is 11 residues long)

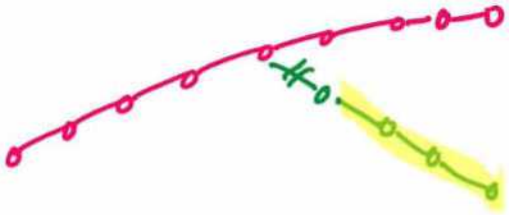


- Note:
  - In case of absence of branching enzymes → spontaneous branching occur after every 24-26 residues

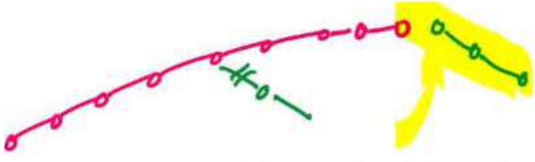
Glycogenolysis



Glycogen phosphorylase (cleaves terminal glucose, but can't act when 4 residues are left at branch site due to stearic hindrance)



Glycogen transferase (transfer the last 3 residues to the adjoining chain)



Branch site glucose exposed

↓

Debranching enzyme

Regulation of glycogenesis and glycogenolysis

00:27:18

- By CcAMP as it regulates glycogen synthase and glycogen phosphorylase
- cAMP affected by insulin, glycogen, epinephrine

Glycogen phosphorylase 00:30:33

- Rate limiting enzyme for glycogenolysis
- Isoenzymes in different tissues
- Similarities and differences in allosteric regulation
- In both liver and muscle:
  - Glucose-6-Phosphate and ATP inhibits glycogen phosphorylase
- In liver but not in muscle
  - Glucose inhibits glycogen phosphorylase
- In muscle but not in liver:
  - AMP and Calcium activates glycogen phosphorylase

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- Note:
  - Receptors for glucagon are absent on muscle, so glycogen cannot be mobilized even when glucagon levels are high
  - Muscle glycogen can be mobilized by epinephrine

GLYCOGEN STORAGE DISEASE

Glycogen storage disease (GSD) 00:00:04

- Deposition of
  - Abnormal type of glycogen
  - Abnormal quantity of glycogen

Features of GSD 00:01:35

- Hypoglycaemia
- Evidence of chronic hypoglycaemia
  - Increase free fatty acids
  - Increase ketone bodies
  - Increase VLDL
  - Increase LDL
  - Increase cholesterol
- Organomegaly

Type of GSD 00:03:30

- GSD-0 → deficiency of glycogen synthase (no synthesis of glycogen)
- GSD-I_a → Von Gierke's disease, Deficiency of Glucose-6-Phosphatase
  - Severe hypoglycemia-exercise causes fainting episodes (because both glycogenolysis and gluconeogenesis are affected due to the deficiency of enzyme)

- Liver, kidney enlarged
- Hyperuricemia (gout, renal stones, tophi)
- Mechanism: Glucose-6-Phosphatase deficiency

↓

Glucose-6-Phosphate increases

↓

Glycolysis get saturated

↓

Upregulated HMP shunt

↓

Increased purine synthesis and breakdown → hyperuricemia

- GSD-II → Pompe's disease, deficiency of acid maltase (lysosomal)

Glycogen in lysosome

↓

Broken into maltose

↓ acid maltase

Glucose

- Variants of Pompe's disease
  - i. Children (hypotonia)
  - ii. Adults
- Multi-organ involvement (cardiomegaly-cause of early death)

- GSD-III
  - Cori's disease, Deficiency of Debranching enzyme

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- Also know as limit dextrinosis (due to presence of limit dextrin on tissue biopsy & microscopic examination)
- Abnormal and highly branched glycogen is deposited
- Severe fasting hypoglycemia
- Glucagon administration in fasting state doesn't increase the blood glucose levels (because glucagon cannot act on debranching enzyme)
- Liver and muscle enlargement

- GSD-IV
  - Andersen disease, Deficiency of branching enzyme
  - Poorly branched glycogen – resemble amylopectin of starch, hence also known as Amylopectinosis
  - Abnormal glycogen
  - Liver and muscle enlargement

- GSD-V
  - McArdle disease, Deficiency of Myo-Phosphorylase
  - Muscle enlargement
  - Exercise intolerance followed by 2nd wind phenomenon

- GSD-VI
  - Hers' disease, Deficiency of Hepatic-Phosphorylase
  - Liver enlargement

- GSD-VII
  - Tarui's disease, Deficiency of PFK-1 in muscle and RBC

PFK-1 deficiency
↓
Glucose increase
↓
Glycogenolysis inhibits
↓
Glycogen storage

- Muscle enlargement
- Exercise intolerance with-out 2nd wind phenomenon

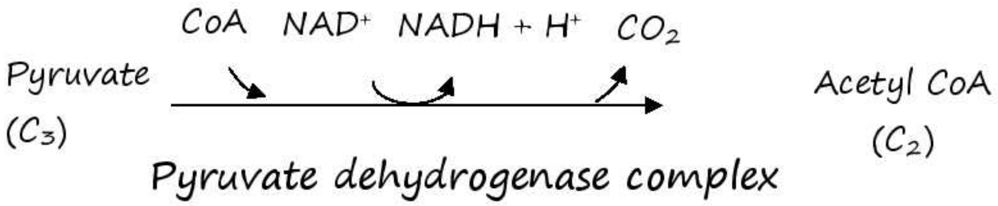
Time frame	3-5 sec	20-30 sec	Before vasodilation (2-5 min)	After vasodilatation
Source of energy for muscle contraction	Stored ATP	Stored Phosphocreatine or Phosphagen (Regenerates ATP)	Glycogenolysis ↓ Glucose ↓ Anaerobic glycolysis	Blood glucose ↓ Aerobic glycolysis
Mc Ardle disease	Correct	Correct	Exercise intolerance (due to ⊖ glycogenolysis)	2nd wind phenomenon
Tarui's disease	Correct	Correct	Exercise intolerance (due to ⊖ glycolysis)	No 2nd wind phenomenon

Management 00:32:00

- GSD-II → Enzyme Replacement Therapy (to prevent early death)
- Other types → Dietary management

LINK REACTION

Links glycolysis and TCA cycle


CoA
NAD$^+$
NADH + H$^+$
CO$_2$
Pyruvate
(C_3)
Acetyl CoA
(C_2)
Pyruvate dehydrogenase complex

Pyruvate dehydrogenase complex 00:02:37

Pyruvate dehydrogenase complex

3 enzymes

1. Pyruvate dehydrogenase
2. Dihydrolipoyl transacetylase
3. Dihydrolipoyl dehydrogenase

5 Co-enzymes

1. Thiamine pyrophosphate (Vitamin B_1)
2. FAD (Vitamin B_2)
3. NAD (Vitamin B_3)
4. CoA (Vitamin B_5)
5. Lipoic acid/ Lipoate

If any block in pyruvate dehydrogenase complex

↓

Pyruvate accumulates in mitochondria

↓

Entry of pyruvate into mitochondria gets blocked

↓

Pyruvate accumulates in cytoplasm

↓

Lactate

↓

Lactic acidosis

Inhibition of pyruvate dehydrogenase complex

00:06:45

- Arsenite
- Mercuric ions
- Dietary deficiency of vitamin B_1 → chronic alcoholics (due to poor dietary intake and alcohol inhibits absorption of vitamin B_1)
- Inherited deficiency of pyruvate dehydrogenase → congenital lactic acidosis

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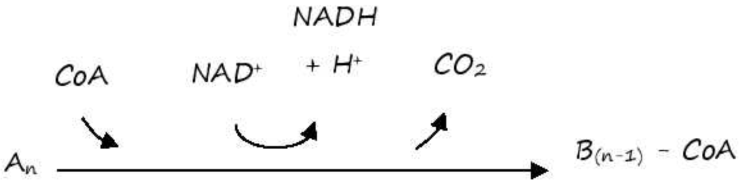
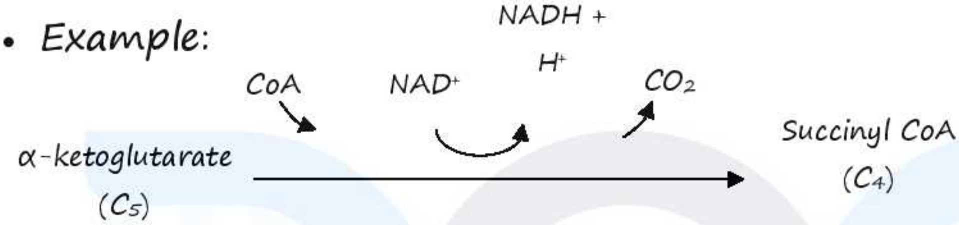
Note

- Pyruvate dehydrogenase complex – similar to two other complex
  - α ketoglutarate dehydrogenase complex (TCA cycle)
  - Branched chain ketoacid dehydrogenase complex (Branched chain amino acid metabolism)

- The above 3 complexes only differ in their 1st enzyme
- Reaction catalysed by these complexs:


CoA
NAD⁺
NADH + H⁺
CO₂
Aₙ
B₍ₙ₋₁₎ - CoA

- Example:

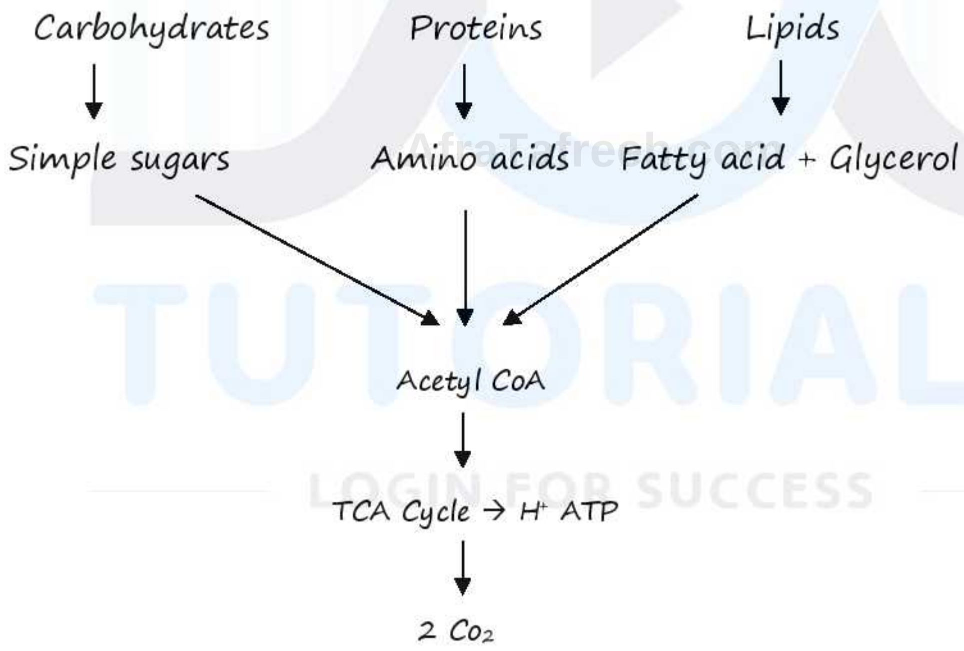

CoA
NAD⁺
NADH + H⁺
CO₂
α-ketoglutarate (C₅)
Succinyl CoA (C₄)

KREB'S CYCLE

Kreb's cycle / TCA cycle / Citric acid cycle

00:00:07

- Final Pathway for all the macromolecules (carbohydrates, lipids, amino acids)
- Described by Hans kreb's (who also described urea cycle)
- TCA cycle – Because 1st product formed is Tri Carboxylic Acid i.e. citric acid


Carbohydrates
Proteins
Lipids
Simple sugars
Amino acids
Fatty acid + Glycerol
Acetyl CoA
TCA Cycle → H⁺ ATP
2 Co₂

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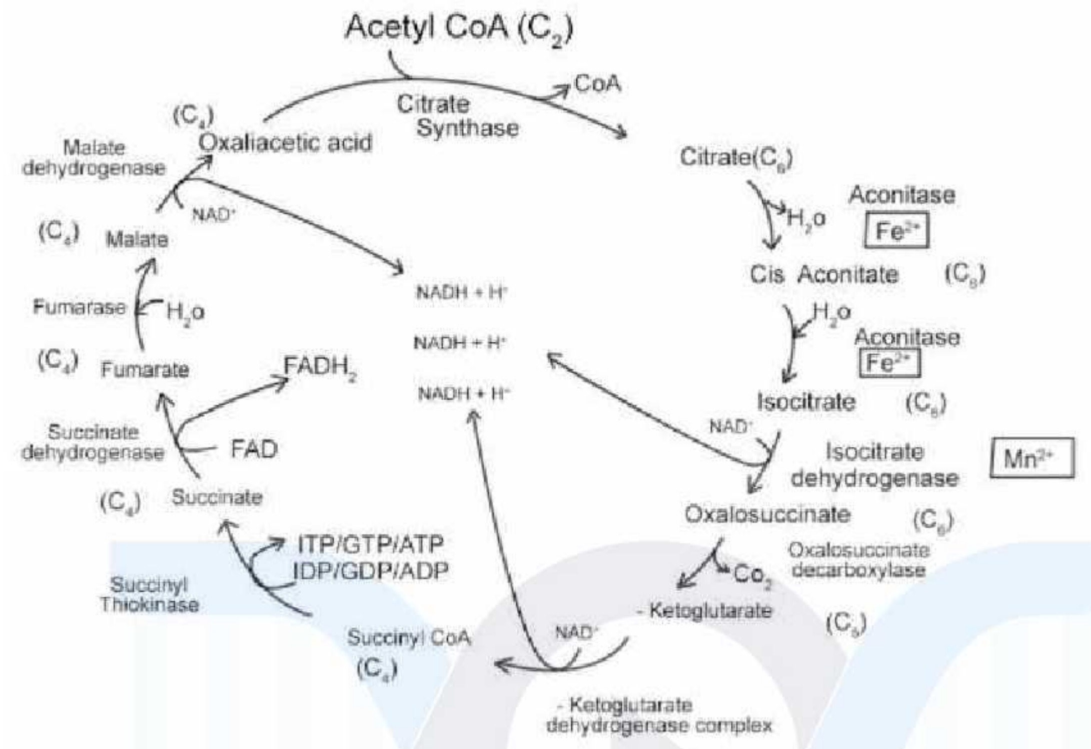
Pathway 00:03:43


Acetyl CoA (C_2)
CoA
Citrate Synthase
Citrate(C_6)
H_2o
Aconitase
Fe^{2+}
Cis Aconitate (C_6)
H_2o
Aconitase
Fe^{2+}
Isocitrate (C_6)
NAD^+
Isocitrate dehydrogenase
Mn^{2+}
Oxalosuccinate (C_6)
Co_2
Oxalosuccinate decarboxylase
- Ketoglutarate (C_5)
NAD^+
- Ketoglutarate dehydrogenase complex
Succinyl CoA (C_4)
ITP/GTP/ATP
IDP/GDP/ADP
Succinyl Thiokinase
(C_4) Succinate
FAD
$FADH_2$
Succinate dehydrogenase
(C_4) Fumarate
Fumarase
H_2o
(C_4) Malate
NAD^+
Malate dehydrogenase
(C_4) Oxaliacetic acid
NADH + H^+
NADH + H^+
NADH + H^+

Note:

- The only reaction in which substrate level phosphorylation occur is succinyl CoA IDP/GDP /ADP ITP/GTP/ATP succinate

Succinyl thiokinase

- Oxalosuccinate decarboxylase is a subunit of isocitrate dehydrogenase.
- Succinate dehydrogenase is considered as a part of complex II of ETC embedded in the inner mitochondrial membrane
- Acetyl CoA can never contribute to gluconeogenesis

Vitamins in TCA cycle: 00:14:57

Vitamin	Source of	Enzymes involved
B1	TPP	α – Ketoglutarate dehydrogenase
B2	FAD	α – Ketoglutarate dehydrogenase succinate dehydrogenase
B3	NAD	α – Ketoglutarate dehydrogenase Isocitrate dehydrogenase malate dehydrogenase
B5	COA	α – Ketoglutarate dehydrogenase

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Energetics 00:17:02

- 3 NADH + H^+ x 2.5 ATP = 7.5
- 1 $FADH_2$ x 1.5 ATP = 1.5
- 1 ATP (Due to subtrate level phosphorylation) = 1.0 (In liver kidney → 1GTP)

10 ATP equivalents net gain/one cycle

Note:

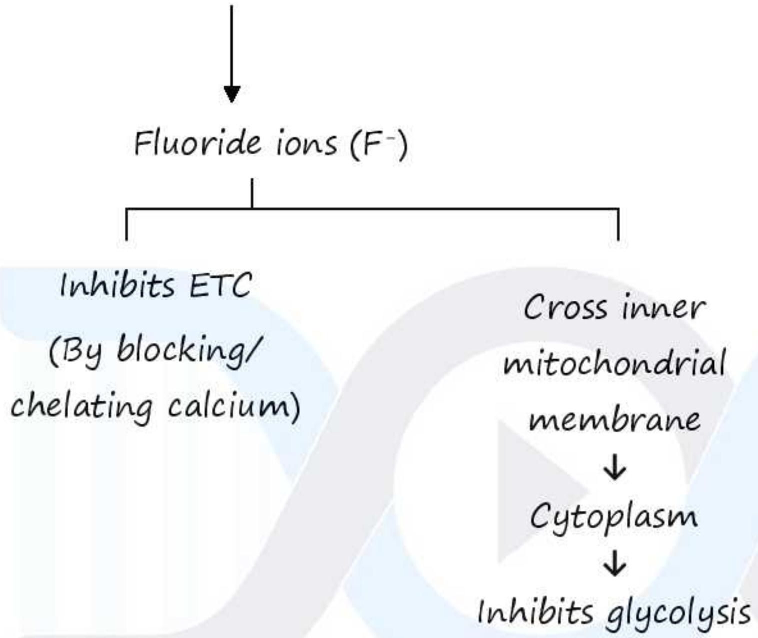
- NADH and $FADH_2$ enter ETC to form ATP by oxidative phosphorylation

Inhibitors 00:19:15

i. Fluoroacetate (Rodenticide)

- Fluoroacetate + Oxaloacetic acid

  Fluorocitrate → Non – competitive inhibitor of aconitase


Fluoride ions (F^-)
Inhibits ETC
(By blocking/
chelating calcium)
Cross inner
mitochondrial
membrane
Cytoplasm
Inhibits glycolysis

- Fluoroacetate when ingested → F released → chelated calcium → hypocalcaemia

ii. Arsenic

- Inhibits α – Ketoglutarate dehydrogenase complex by non-competitive inhibition of lipoic acid
- Arsenic reacts with – SH group of lipoic acid

iii. Malonate

- Competitive inhibitor of succinate dehydrogenase due to structural similarity with succinate

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ELECTRON TRANSPORT CHAIN

- Electron Transport Chain (ETC) also known as Respiratory chain
- ETC gets the reducing equivalents from NADH + H^+ and $FADH_2$ and converts them into ATP
- ETC – 67 proteins
  - 13 by mitochondrial DNA (<20% of total proteins)
  - 54 by nuclear DNA
- The 67 proteins are clustered in 4 complexes which are embedded in Inner mitochondrial membrane projecting towards matrix
  - Complex I – NADH-Q Oxidoreductase
  - Complex II – Succinate-Q Reductase
  - Complex III – Q-cytochrome c oxidoreductase
  - Complex IV – Cytochrome c oxidase

- Only 2 subunits are mobile (responsible for movement of electrons from one complex to other complex)
  - Substance Q/ Ubiquinone
  - Cytochrome C

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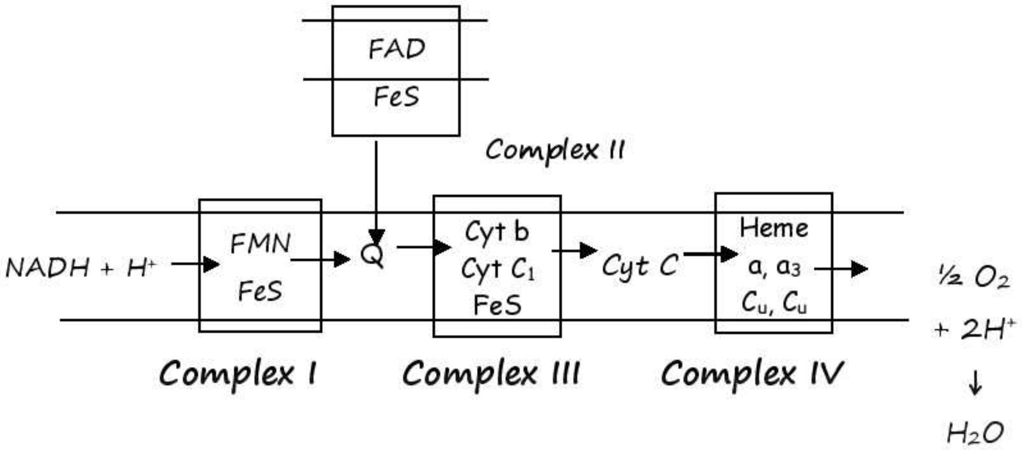
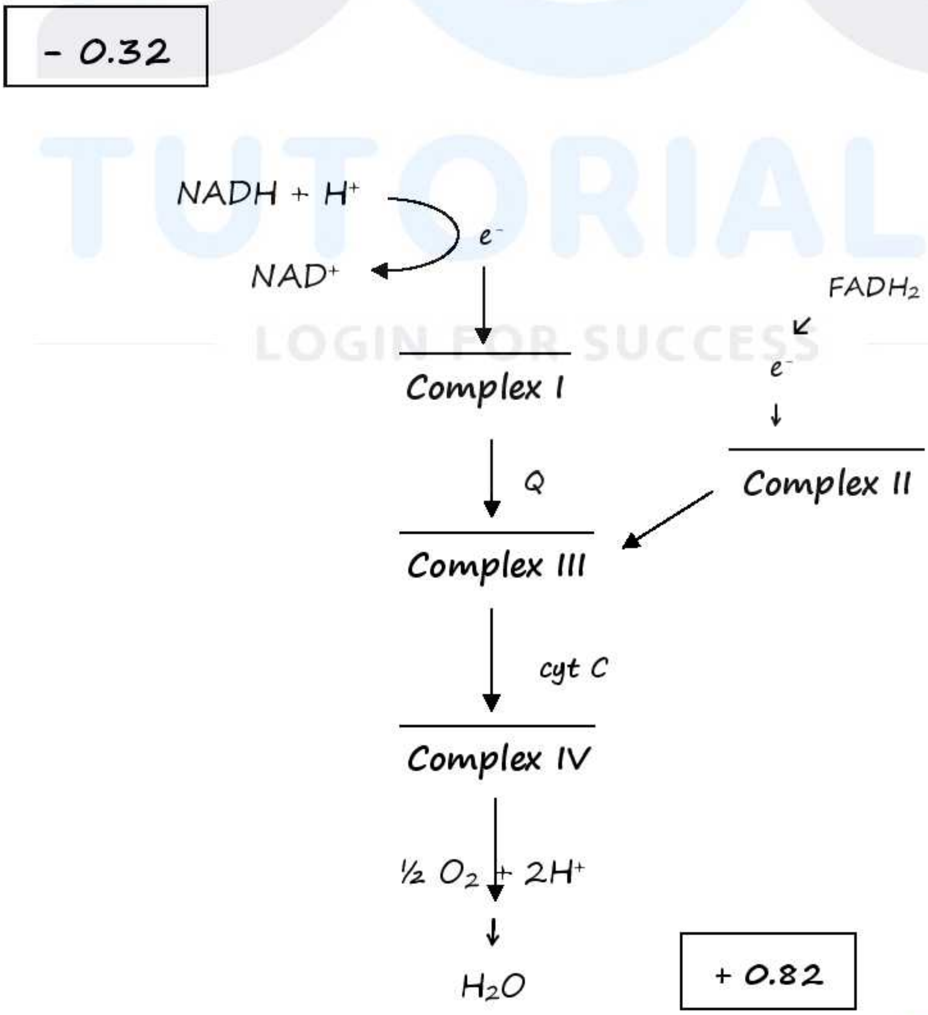
Composition of ETC 00:05:57

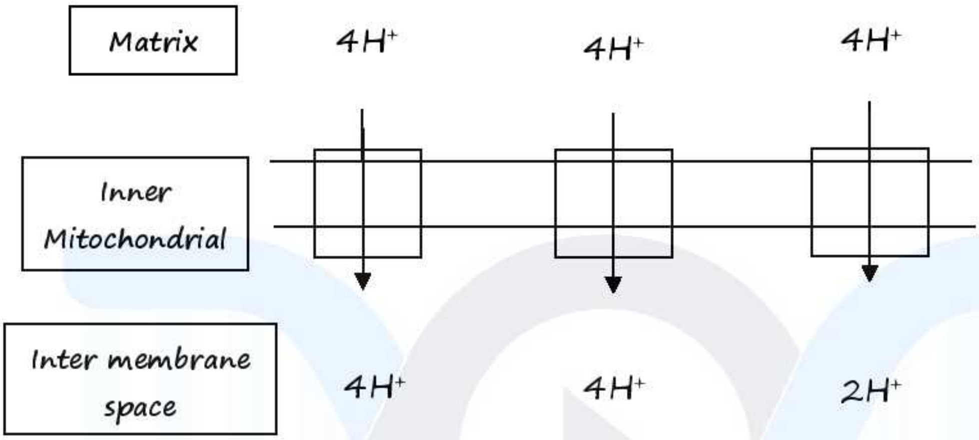

FAD
FeS
Complex II
NADH + H⁺
FMN
FeS
Q
Cyt b
Cyt C₁
FeS
Cyt C
Heme
a, a₃
Cu, Cu
½ O₂
+ 2H⁺
H₂O
Complex I
Complex III
Complex IV

- Movement of e⁻ is as per redox potential i.e., from higher redox (negative value) to lower redox (positive value)


- 0.32
NADH + H⁺
e⁻
NAD⁺
FADH₂
e⁻
Complex I
Q
Complex II
Complex III
cyt C
Complex IV
½ O₂ + 2H⁺
H₂O
+ 0.82

- Movement of e$^-$ from
  - C-I to C-II
  - C-II to C-I
  - C-III to C-I
  - C-IV to C-III

  not possible because e$^-$ moves from higher redox to lower redox potential

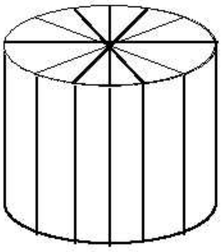

Matrix
4H$^+$
4H$^+$
4H$^+$
Inner Mitochondrial
Inter membrane space
4H$^+$
4H$^+$
2H$^+$

- NADH = 10H$^+$ transported → $\frac{10H^+}{4H^+/ATP}$ = 2.5 ATP
- FADH = 6H$^+$ transported → $\frac{6H^+}{4H^+/ATP}$ = 1.5 ATP

4 H$^+$ required to generate 1 ATP

F_1 particle or ATP synthase or complex – V

00:20:13



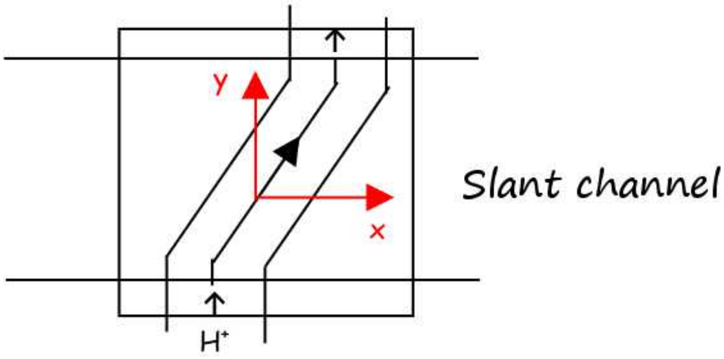
F_0

- Embedded in Inner Mitochondrial membrane
- Disc like structure
- Made up of multiple 'C' subunits → acts as channel for H^+ transport from Inter membrane space to matrix

F_1

- α subunit → holds β subunits together (packing)
- β subunits
  - ATP synthase ($ADP + P_i \rightarrow ATP$)
  - Catalytic unit
- γ subunit → stalk
- δ subunit → stabilizes whole complex

Cross-section of 'c' subunit



- H^+ travels at an angle
- Movement along Y-axis → pushes the H^+ forward
- Movement along X-axis → acts on the walls

↓

rotation of F_0 subunit

↓

Through γ^1 stalk rotation transmitted to F_1

↓

Movement utilized by β subunits

↓

Brings ADP + Pi together

↓

ATP

- Potential energy of H^+

↓

Kinetic energy of 'C' subunit

↓

ATP synthesis

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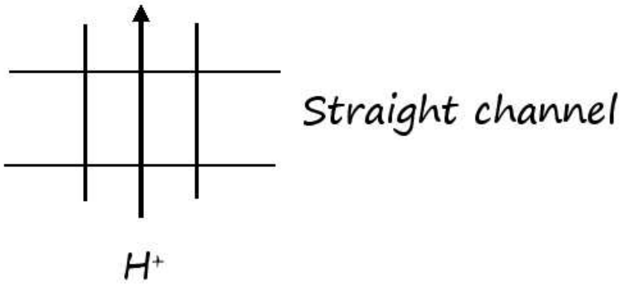
Uncoupler protein (UCP)



- Opened by uncouplers → uncoupling
- All potential energy of H^+ is released as heat

Oxidative phosphorylation

- Oxidation:
  - NADH → NAD^+
  - ½ O_2 → H_2O
- Phosphorylation: ATP formation
- Oxidation – occur in complexes I, II, III, IV
- Phosphorylation – occur in F_o – F_1 particle

} Coupled

Uncoupling

- Oxidation occur but phosphorylation do not occur
- Uncouplers
  - Endogenous – acts on specific type of UCP
  - Exogenous – non-specific i.e., acts on various types of UCP

- UCP types
  - 1
  - 2

  - 3
  - 4

Inhibitors of ETC 00:36:53

- **Complex I:**
  - Pericidin A
  - Amobarbital
  - Amytal
  - Rotenone
  - Secobarbital
  - Chlorpromazine
  - Guanethidine
  - Demerol
- **Complex II:**
  - Malonate
  - Carboxin
- **Complex III:**
  - BAL (British Anti-Lewisite)
  - Antimycin A
  - Phenformin/ Metformin

MALA (Metformin Associated Lactic Acidosis)

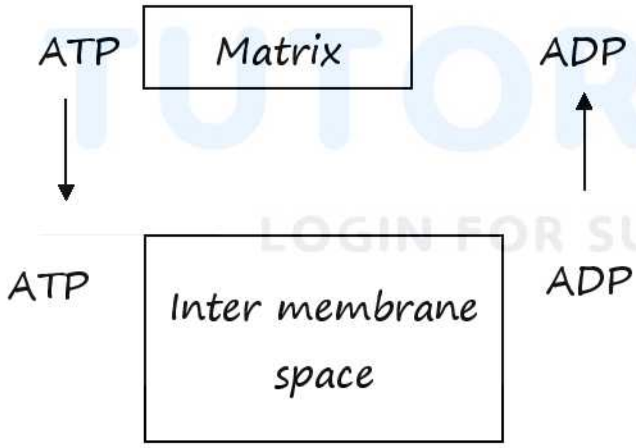
- Due to unmonitored intake of metformin
- Metformin mainly excreted by kidney, so if kidney function deteriorates, metformin accumulates

- Complex IV:
  - Hydrogen sulphide
  - Carbon Mono-oxide
  - Cyanide
  - Azide

- Complex V
  - F_o → Oligomycin
  - Venturicidin/ Aabomycin
- F_1 → Tentoxin
  - Efrapetin

ADP-ATP Translocase inhibitor:

- Atractyloside


ATP
Matrix
ADP
ATP
Inter membrane space
ADP

Uncouplers: 00:43:26

i. Exogenous
  - 2,4 – Dinitrophenol
  - Dicumarol

ii. Endogenous

- UCP 1: Thermogenin
- (present only in brown adipose tissue)
- UCP 3:
  - Thyroxine hormone
  - Non-Epinephrine
  - Epinephrine
  - Leptin
- UCP 4:
  - Essential fatty acid deficiency
  - Purine nucleotides

Note:

- Bilirubin is a non-specific uncoupler

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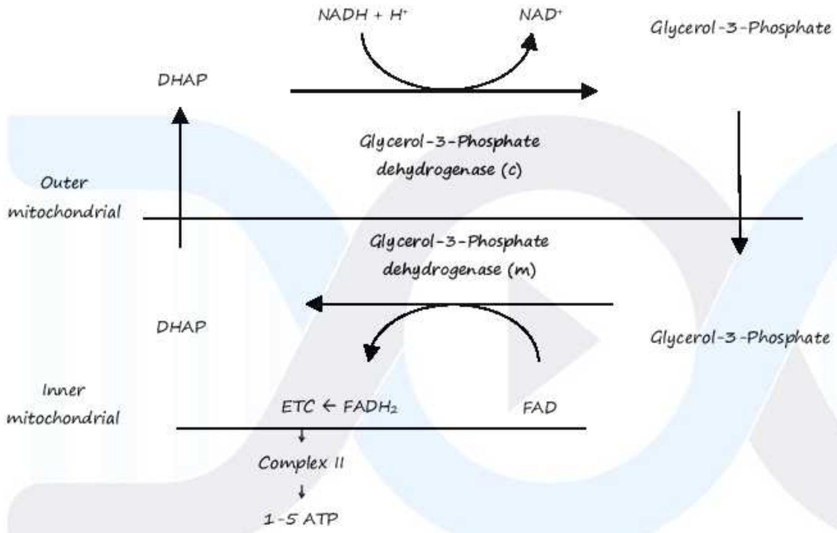
SHUTTLERS

Glycerol-3-Phosphate shuttle 00:01:10

- Transfer reducing equivalents from cytoplasm to mitochondria
- Mainly operative in CNS, skeletal muscles


NADH + H⁺
NAD⁺
Glycerol-3-Phosphate
DHAP
Glycerol-3-Phosphate dehydrogenase (c)
Outer mitochondrial
Glycerol-3-Phosphate dehydrogenase (m)
DHAP
Glycerol-3-Phosphate
Inner mitochondrial
ETC ← FADH₂
FAD
Complex II
1-5 ATP

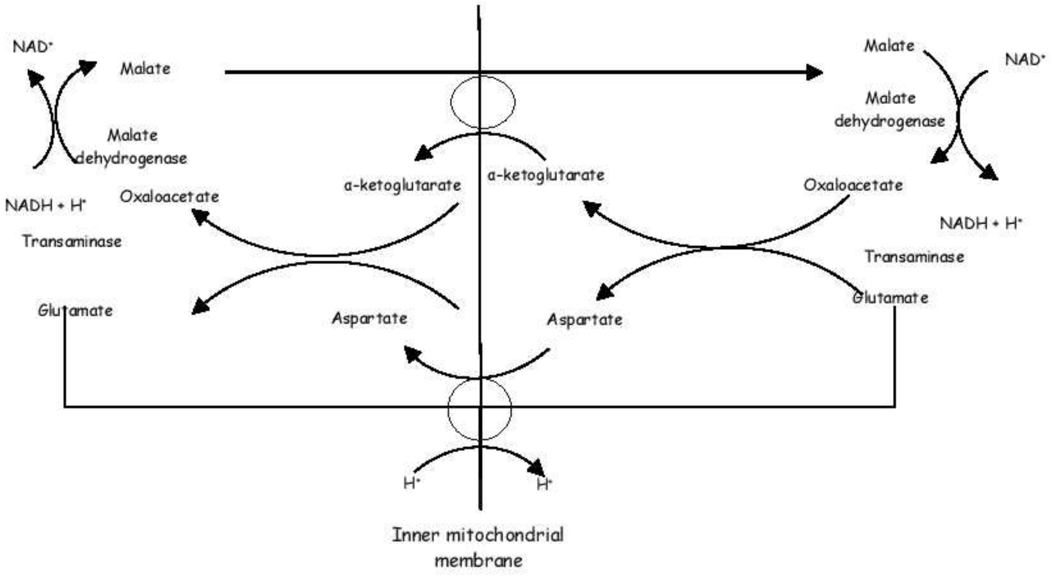
(c) – cytosolic isoenzyme

(m) – mitochondrial isoenzyme

- Only one set of molecules are involved
- Very fast release of energy (no transporter required)

Malate Aspartate Shuttle 00:06:40

- Transfer reducing equivalents from cytoplasm to mitochondria
- Mainly operative in liver, kidney


NAD⁺
Malate
Malate dehydrogenase
NADH + H⁺
Oxaloacetate
Transaminase
Glutamate
α-ketoglutarate
α-ketoglutarate
Aspartate
Aspartate
Malate
NAD⁺
Malate dehydrogenase
Oxaloacetate
NADH + H⁺
Transaminase
Glutamate
H⁺
H⁺
Inner mitochondrial membrane

- 2 transporters are required
- Generates 2.5 ATP

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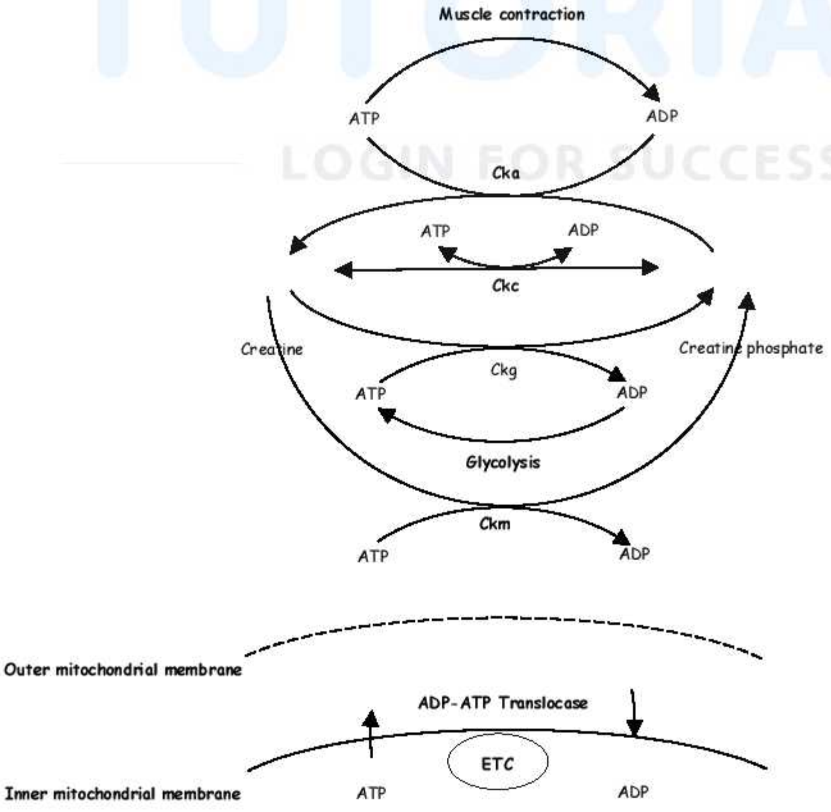
Creatine phosphate shuttle 00:13:12

- Maintains creatine phosphate pool which replenishes ATP when required


Muscle contraction
ATP
ADP
Cka
ATP
ADP
Ckc
Creatine
Creatine phosphate
Ckg
ATP
ADP
Glycolysis
Ckm
ATP
ADP
Outer mitochondrial membrane
ADP-ATP Translocase
ETC
Inner mitochondrial membrane
ATP
ADP

- Most important source of ATP to convert creatine to creatine phosphate is ATP from mitochondria

INTRODUCTION TO CLASSIFICATION OF ENZYMES

Enzymes 00:00:10

- What is the composition – proteins mostly.
  - Ribozymes (an exception) → these are RNA with catalytic activity

    Example: -
    - Peptidyl transferase

      ↓

      Prok 23s γRNA

      Euk 28s γRNA
    - Small nuclear RNA [snRNA]

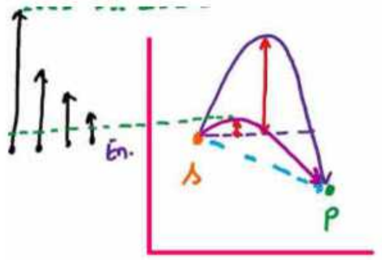
      Involved in splicing
    - Introns – self splicing activity
- What are its functions?

  Bio-catalyst

  ↓

  Substance which modifies the rate of reaction

  Decrease energy of activation



- Catalysts is a substance which decreases the energy of activation
- Catalyst increase the rate of reaction
- So, equilibrium is achieved faster
- Concentration of substrate and product at equilibrium of catalyzed and uncatalyzed reaction is same

- Enzyme unit is described by
  1) Amount of substrate consumed per unit time

     (or)

  2) Amount of product formed/unit time
  3) IU/L

Classification of enzymes 00:12:20

- It is given by international union of bio chemistry and molecular biology
- It is divided under:
  - Class
  - Sub class
  - Sub-sub-class
  - Functions

  Previously is 6 classes now 7 classes are described 'OTHLILT'

I. Oxido reductase 00:16:07

- Oxidation / reduction
- Addition/ removal of electron/H+ /O2

#Dehydrogenase

#Oxygenase (Hydroxylases)

Mono oxygenase

Dioxygenase

Mixed function oxygenase

#Peroxidase: H2O2 is present

II. Transferase 00:19:01

#Methyl transferase

#Amino transferase

#keto transferase

#Phosphotransferase →some type

- Kinase – transfer po4 to / from ATP
- Phosphorylase – inorganic po4 [pi] is involved.

III. Hydrolase 00:20:54

Hydrolase: breaks bonds using H2O (hydrolysis)

#Amylase

#Lipase

#Protease

#Peptidase

#Nuclease

#Nucleotidase

#Nucleosidase

#Phosphoblesterare

#Phosphatase

Note: phosphatase is different from phosphorylase where, in latter transfer of po4 happen where as in former cleavage using the reaction of Hydrolysis.

IV. Lyase 00:23:01

Lyase: breaks bonds without H2o usage

#Decarboxylase – releases out CO2 [requires vitamin B6]

#Aldehyde – releases out aldehyde

#Carbonic anhydrase

V. Isomerase 00:24:40

Isomerase: able to interchange configuration of molecules without adding or remaining anything

#Epimerase – interchange the epimers

#Racemase – interconversion of L and D isomers

#Mutase – transfers groups from one place to another place within a molecule

Example: Phosphoglucomutase.

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VI. Ligase 00:26:09

Ligase: joins the molecules

#Carboxylase: Co2 is substrate

[requires coenzymes like vitamin B7/biotin]

#Synthelase: ATP dependent reaction

Here when ATP is broken down into ADP and phosphate,

Energy is liberated and it is used up for reaction.

VII. Translocase 00:27:49

Translocase: these will transfer molecules across membranes from one side to another.

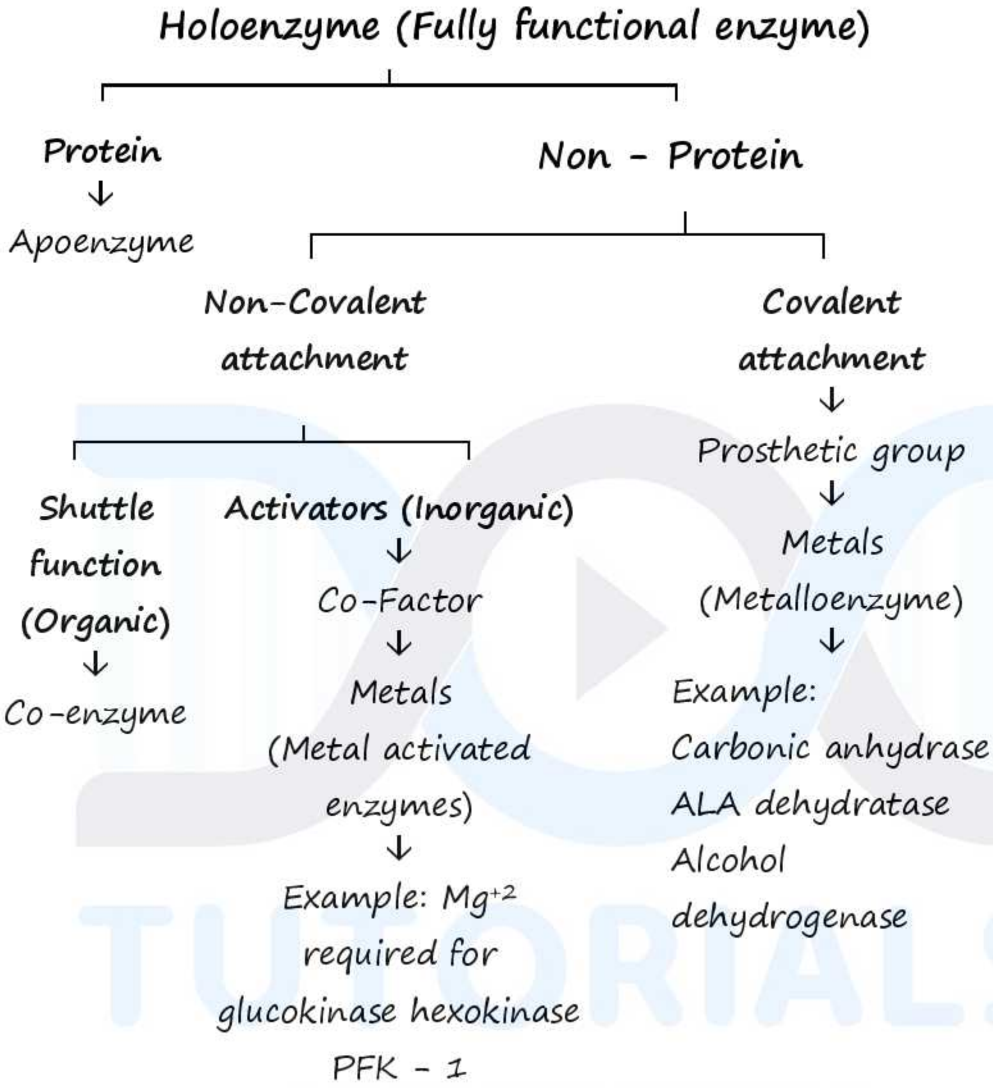
#ADP-ATP Translocase.

Note: Difference between kinase and synthetase:

Both enzymes are involved with ATP but kinase is where po4 is transferred synthetase is where liberated energy is utilized.

ENZYME - PROPERTIES

Constituents of enzyme: 00:00:22


Holoenzyme (Fully functional enzyme)
Protein
Apoenzyme
Non - Protein
Non-Covalent attachment
Covalent attachment
Prosthetic group
Metals
(Metalloenzyme)
Example:
Carbonic anhydrase
ALA dehydratase
Alcohol dehydrogenase
Shuttle function (Organic)
Co-enzyme
Activators (Inorganic)
Co-Factor
Metals
(Metal activated enzymes)
Example: Mg+2 required for glucokinase hexokinase PFK - 1

Properties of enzymes 00:04:39

i. Active site – Site where
  - Substrate attaches
  - Catalysis occur
  - Product forms

- Enzymes are named by

  - Substrate and catalysis → ex: Hexokinase substrate catalysis
  - Product and catalysis → ex: DNA polymerase product catalysis
- Name of the enzyme describes the primary function of enzyme

ii. Catalytic efficiency

- Denoted by Kcat/Km (Kcat →reaction constant)
- Vmax = Maximum initial velocity (Vi) of reaction
- Vmax is achieved when ideal conditions are provided
  - Optimum PH
  - Optimum temperature
  - Substance concentration (S) = ∞ (Non – limiting)

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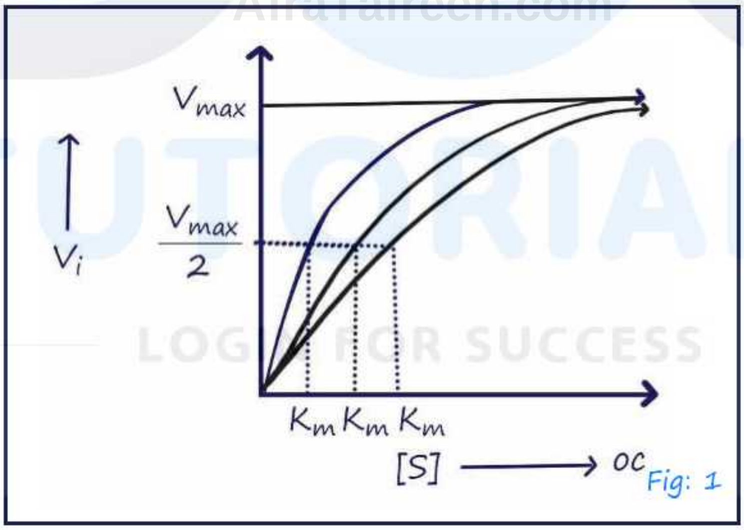


Fig: 1

- Km = [S] at Vmax/2
- Vmax = Calculated value
- Km → Affinity between enzyme and substrate
- Km α 1/Affinity
  - Low km = High affinity

  - High km = Low affinity

iii. Specificity

- It is a spectrum; most enzymes are in between the below 2 extremities
- Lock and key model – exact fit
- Induced fit model – no fitment, shapes are malleable

Additional properties 00:18:30

i. Allosteric site (Allosteric enzymes)

- Site other than active site – molecules attach non-covalently
  - No catalysis
  - Modification/alternation of catalytic efficiency (Vmax ↑/↓, Km ↑/↓)

ii. Covalent modification

- Covalent attachment of molecule
- Effect – modification of catalytic efficiency

iii. Cofactors/Coenzymes

- Required for some enzymes

TUTORIALS LOGIN FOR SUCCESS

MACHANISM OF CATALYSIS

- Enzymes decrease energy of activation by
  - i. Catalysis by proximity
  - ii. Acid base catalysis
  - iii. Catalysis by strain
  - iv. Covalent catalysis

i. Catalysis by proximity 00:00:56

- Role in when interaction between two molecules is involved
- Enzymes provided attachment point for substrates, so that they are close – by and the reaction occurs

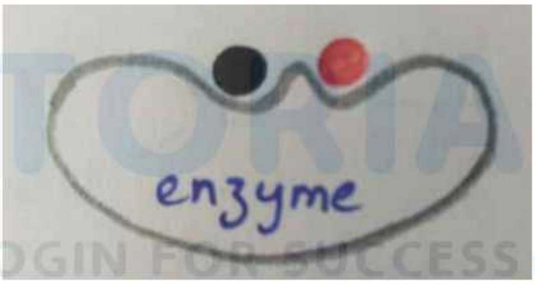


Fig: 1

ii. Acid Base catalysis: 00:02:58

- One part of enzyme acts as acid and other part as base
- Modifies substrate
- Product

- Example: Aspartic protease family (Pepsin, cathepsin, HIV protease)

iii. Catalysis by strain 00:04:54

- Cleaves a molecule to give two or more different products
- Achieved by conformational change of enzyme on attachment of the substrate

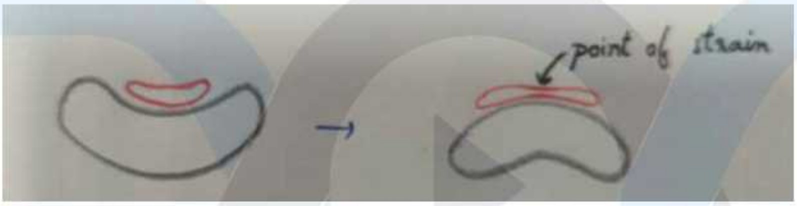
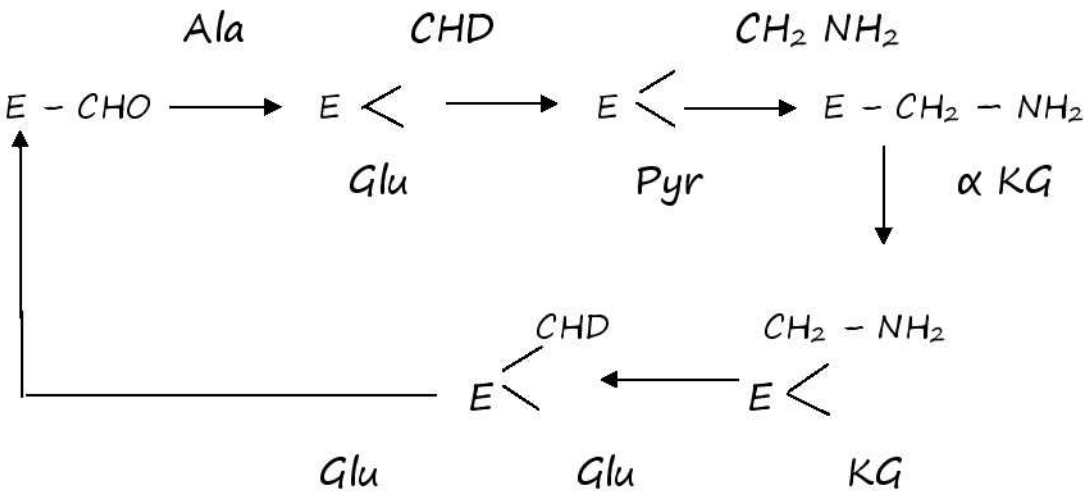


Fig: 2

iv. Covalent catalysis 00:06:45

- Transient enzyme structure modification covalently
- Examples: Serine protease family (trypsin, Proteas elastase, Chymotrypsin) Fructose – 2,6 – Bisphosphatase
- Ping pong reaction: During transamination


Ala
CHD
CH₂ NH₂
E – CHO
E
E
E – CH₂ – NH₂
Glu
Pyr
α KG
CHD
CH₂ – NH₂
E
E
Glu
Glu
KG

- Ala → Alanine
- Pyr → Pyruvate
- α KG → α Ketoglutaric acid
- Glu → Glutamate
- $E - CHO$ → Enzyme (Transaminase
- $E - CH_2 - NH_2$ → Modified enzyme

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KINETICS

Factors affecting reactions velocity

i. Substrate concentration [s] → hyperbolic curve

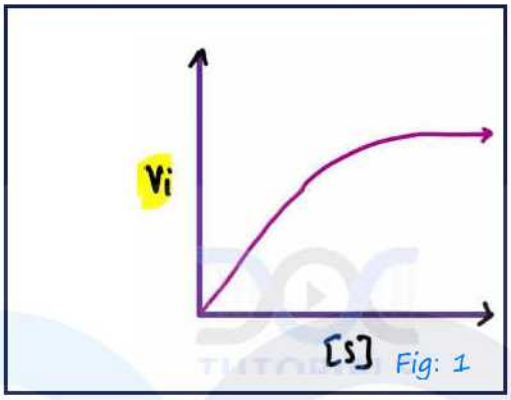

Fig: 1

- After certain time, increase in [s] does not cause increase in vi → plateau phase
- Plateau phase is typically achieved at 5-10 times of km
- Q. If (s) = 1000km, what will happen to reaction velocity if [s] increased further
- Ans: No change in reaction velocity (due to plateau phase)

ii. Effect of temperature/ pH --. Bell shaped curve

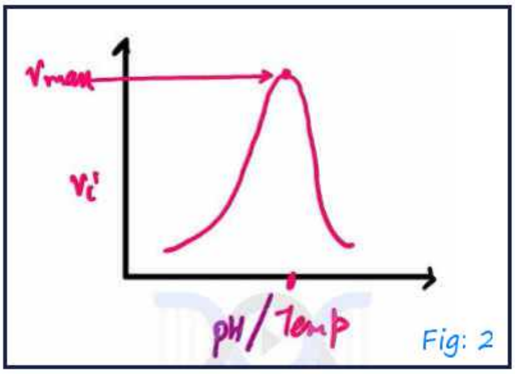

Fig: 2

iii. Effect of enzyme concentration (E) → Linear curve

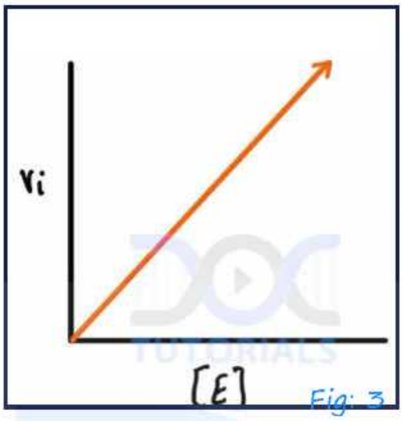

Fig: 3

If (E) increase → velocity increase
If (E) decrease → velocity decrease

- Q. If with certain [E], certain amount of product formed in 9 minutes. How much time is required to produce the same amount of product, if (E) is decreased to (E)/3
- Ans: 27 minutes

Enzyme kinetics 00:07:37

- Described as Zero order, first order, second order reactions
- Reactions following 1st order kinetics are described by Michaelis Menten equation
- **Michaelis Menten equation:**

  $Vi = Vmax [s] / Km^{+} [s]$

- Good for studying single reaction
- Not suitable for comparison because of curved slope

- Michaelis Menten equation can be modified to get straight line graph in which slope is constant, hence can be used for comparison

$$\frac{1}{Vi} = \frac{K_m + (S)}{V_{max}\,(S)}$$

$$\frac{1}{Vi} = \frac{K_m}{V_{max}} \times \frac{1}{(s)} + \frac{(s)}{V_{max}\,(s)}$$

$$\frac{1}{Vi} = \frac{K_m}{V_{max}} \; \frac{1}{(s)} + \frac{1}{V_{max}}$$

y = m x + c → equation for straight line graph

y ↓ Dependent variable

m ↓ constant

x ↓ variable

c ↓ constant

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Line weaver and Burke plot/ Double reciprocal plot

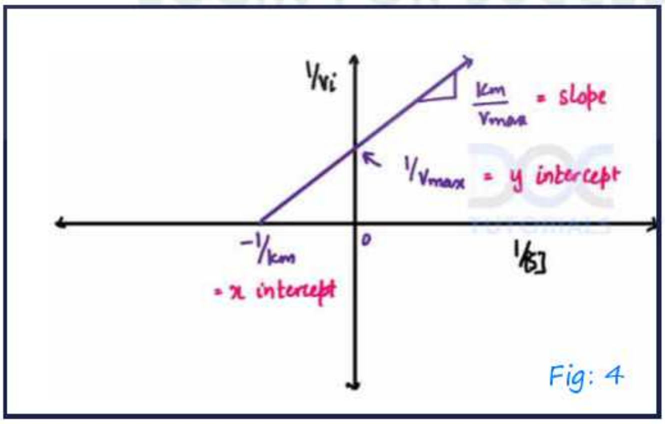

Fig: 4

- Disadvantage:
  - Clustering i.e., different graphs coming together in a short area on x and y axis

Single Reciprocal plots

- Used when clustering on double reciprocal plot
- Eadie Hofstee plot (Vi vs Vi/(s))
- Hanes Woolf plot ((s)/Vi vs (s))

Dixon plot

- Used In pharmacology to compare potency of competitive inhibitors

ENZYME - INHIBITION

Enzyme's inhibition: 00:00:20

i. Reversible

ii. Irreversible

Reversible Inhibition 00:02:26

Competitive

- Inhibitor attaches at active site and has similar structure as substrate
- Km ↑ (as affinity ↓)
- Vmax: Normal (Because at Vmax, (S) = ∞ so the substrates displace the inhibitors)

$$\begin{tikzcd} E + S \arrow[r, shift left] & ES \arrow[l, shift left] \arrow[r] & P \\ + & & \\ I \arrow[d] \arrow[uur, "\text{At } V_{max}"'] & & \\ EI & & \end{tikzcd}$$

- Ex: ACE inhibitors Malonate vs succinate for succinate dehydrogenase

Mixed

- Inhibitor attaches to allosteric site and it is not similar to substrate
- Vmax ↓ (Because some enzymes do not participate)
- Km depending on inhibitor

↑ Normal ↓

↓

Non-competitive inhibition

$$\begin{tikzcd} E + S \arrow[r, shift left] \arrow[d, "+ I"] & ES \arrow[l, shift left] \arrow[r] \arrow[d, "+ I"] & P \\ EI + S \arrow[r] & ESI \arrow[ur, "\times"] & \end{tikzcd}$$

- Ex: Lead inhibits ferro chelatase and ALA dehydratase

Uncompetitive

- Inhibitor attaches to enzymes – substrate complex
- Vmax ↓ (Because some enzymes do not participate)
- Km ↓ (Because the affinity increases as the forward reaction speeds up to meet equilibrium)

$$\begin{tikzcd} E + S \arrow[r, shift left] & ES \arrow[l, shift left] \arrow[r] \arrow[d, "+ I"] & P \\ & ESI \arrow[ur] & \end{tikzcd}$$

- Ex: Phenylalanine inhibits Alkaline phosphate

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Clinical correlation:

i. In methanol poisoning ethanol is used in treatment

Mechanism: Methanol poisoning ← Ethanol

Reduces the action of alcohol dehydrogenase by competitive inhibition

↓ Alcohol dehydrogenase

Formaldehyde, formic acid

↓

Damages optic nerve (Blindness) and death

ii. Digitalis poisoning

- As there is no competitive inhibitor for digitalis it should be removed from circulation by using antibodies (Digibind) or dialysis

Changes on Lineweaver burk plot:

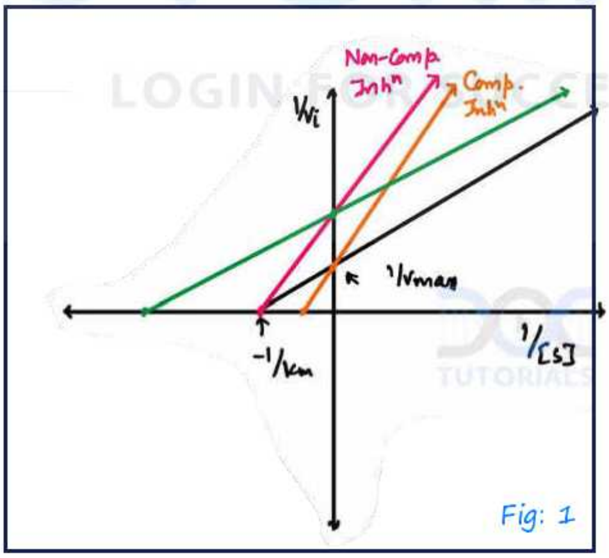

Fig: 1

Note:

Type of inhibition	Relation with normal reaction graph
Competitive inhibition	Y intercept same
Non – Competitive inhibition	X Intercept same
Uncompetitive inhibition	Both X and Y intercepts are different

Irreversible inhibition 00:22:13

i. group specific reagents → DIPF (Insecticide) acts on chymotrypsin and Acetylcholinesterase

ii. Reactive subtrate analogues/affinity labels → Bromoacetal phosphate inhibits Triose phosphate Isomerase (TPI) → TRCK (Insecticide) acts on chymotrypsin

iii. Suicide inhibitors/Mechanism based inhibitors:

- Dimethyl propargyl amine (DMPA) acts on monoamine oxidase (MAO)
- Penicillin acts on glycopeptide transpeptidase
- Aspirin acts on thymidylate synthase
- Allopurinol acts on xanthine oxidase

Mechanism: Allopurinol

↓ Xanthine oxidase

Oxypurinol/Alloxanthine

Attaches irreversibly at active site

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- Allopurinol toxicity – Secondary inhibitor of orotidylate decarboxylase (Enzyme 6 of pyrimidine synthesis pathway)

ENZYME REGULATION

Enzyme regulation 00:00:23

- By changing enzyme activity
- By changing enzyme quantity

Long term Control 00:01:56

- Genetic level (Long term control) – Takes hours to days
- Induction
- Repression and depression
- Degradation

Middle term control 00:04:30

- Type II hormones interact with receptors at plasma membrane (Mid-term control) – Takes minutes to hours
- Covalent modification
- Activate 2nd messengers

Short term control 00:06:20

- Substances (Short term control) – Takes seconds to minutes
- Short term control is active only for allosteric enzymes

- Allosteric enzymes
  - Effectors (Not part of metabolic pathway in which enzymes acts ex; PFK 1 (Glycolysis) limited by citrate (Kreb's cycle)
  - Positive effect
  - Negative effect
  - Feedback inhibition – ex: Glucose 6 phosphate acting on hexokinase → Molecules from the metabolic pathway affecting the enzyme
  - Feed forward activation – ex: Fructose 6 phosphate acting on PFK - 1

ISOENZYMES

Isoenzymes:

- Different proteins

i. With same catalytic activity

ii. Which catalyze same reaction

Properties that may differ

- Km
- Vmax
- Hormonal regulation
- Allosteric regulation
- Structure
- Electrophoretic mobility
- Immunological identity or response
- Part of different pathways
- Tissue specific
- Location in different compartments of a cell

Examples:

Hexokinase	Parameter	Glucokinase
↓ (More affinity for glucose)	Km	↑ (Less affinity for glucose)
↓	Vmax	↑
All tissues	Location	Liver, β cells of pancreas
Not affected	Hormonal regulation	↑ in enzymes concertation (Activity not ↑)
(–) By glucose 6 phosphate	Allosteric regulation	Nil
Glycolysis	Pathways	Glycolysis (β – cell); Glycogenesis (Liver)

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Carbamoyl Phosphate synthase: I & II 00:08:56

CPS I	Parameter	CPS II
Urea cycle	Pathways	Pyrimidine synthesis
Mitochondria	Compartment	Cytoplasm
Co_2 + NH_3/NH^+	Substrates	Co_2 + Glutamine (Donor of NH_3)
N-Acetyl glutamate (NAG)	Co-enzymes	Not required
2 ATP	Energetics	1 ATP

Lactate Dehydrogenase (LDH) 00:12:35

- LDH made up of 4 polypeptides, coded by 2 genes: H & M
- 5 different LDH Based on polypeptide composition

LDH I	HHHH (H4)	Heart
LDH II	HHHM (H3M1)	RBC
LDH III	HHMM (H2M2)	Non-specific
LDH IV	HMMM (H1M3)	Liver and muscle
LDH V	MMMM (M4)	Liver and muscle

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Creatine kinase (CK)/Creatine phosphokinase (CPK) 00:15:25

- CPK made up of 2 polypeptides coded by 2 genes M & B
- 3 different CPK based on polypeptide composition

CPK I	BB	Brain	< 1% in blood
CPK II	MB	Heart	~ 6 to 15% in blood
CPK III	MM	Skeletal muscle	~ 85% in blood

Note:

- Isoenzymes are used to localize the tissue under stress/damage

APPLICATIONS OF ENZYMES

Clinical applications 00:00:30

1.Diagnosis of time damage

- Most enzymes in blood are non-functional: they are due to tissue turnover

Functional enzymes in plasma:

- Pseudocholinesterase
- Butyryl cholinesterase
- Activated clotting factors
- Activated complement factors
- Protein C and S
- Lipoprotein lipase (Acts on plasma substrate, attached to capillary walls)

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Cardiac profile:

- CPK – MB
- AST
- LDH

Liver profile

- AST, ALT → ↑ in liver damage
- Alkaline phosphatase
- 5 nucleotidase ↑ In canalicular block (Obstructive jaundice)
- γ – glutamyl transpeptidase

Pancreatic profile:

- Amylase
- Lipase
- Tumor markers
- Acid phosphate (Prostate cancer)

2.Analytical role in laboratory – helps in estimating the levels of different analytes

- Glucose – Glucose oxidase + peroxidase
- Cholesterol – Cholesterol esterase, lipase
- Uric acid – Urease
- Elisa – Peroxidase, alkaline phosphate (Enzymes linked Immunosorbent assay)

3.Therapeutic role

- Streptokinase/Urokinase – Arterial blockage
- Chymotrypsin – break pus loculi
- Serratiopeptidase – Prevent swelling subsequent to inflammation
- Pancreatic enzyme – In case of diseased pancreas
- Enzyme replacement therapy for inborn errors of metabolism
- Gaucher's disease (1st disease for which enzyme replacement therapy is available)
- Fabry's disease
- Pompe's disease
- MPS – IH (Hurler disease)

- IHS (Hurler scheic disease)
- IS (Hunter disease)
- MPS – II (Hunter disease)
- MPS – VI (Maroteaux – lamy disease)
- Ada – SCID

Non – clinical applications 00:12:35

1. Food industry – Papain used for meat softening
2. Garment industry – Enzymes digest the fabric there by increase the shine of old garments
3. Petroleum industry – Enzymes clear petroleum spills

Rate limiting enzyme 00:13:34

- Most important rate limiting enzyme (Because a pathway can have more than one rate limiting enzyme)
- Rate limiting enzyme → Enzyme catalysing irreversible reaction and the product should be used only for the same pathway

Pathway/Process	Most important rate limiting enzyme
Glycolysis	PFK – 1
Gluconeogenesis	Fructose – 1,6 Bis/Diphosphatase
TCA cycle	Isocitrate dehydrogenase (Oxalosuccinate decarboxylase)
Glycogenesis	Glycogen synthase
Glycogenolysis	Glycogen phosphorylase
HMP shunt	G6PD
Purine synthesis	CPS III
Urea cycle	CPS I
Fatty acid synthesis	Acetyl CoA carboxylase
Fatty acid oxidation	Carnitine acyl transferase – I or Carnitine palmitoyl transferase – I (CPT – I)
Ketogenesis	HMG CoA Synthase
Cholesterol Synthesis	HMG CoA reductase
Bile acid synthesis	> – α – Hydroxylase
Urine acid synthesis	Xanthine oxidase
Catecholamine synthesis	Tyrosine hydroxylase

SPHINGOLIPIDOSES

- Ceramide containing complex lipids accumulate in cells (neurons)
  ↓
  Neurodegeneration
  ↓
  Shortening of life span
- Rate of synthesis is normal
- Enzymatic defect in lysosomal degradation pathway → type of lysosomal storage disorders
- Extent of defect is same in all tissues
- Genetic defect: Autosomal recessive (Inheritance pattern)
  exception: Fabry's disease → X linked recessive
- Cherry red spot on retinal examination in all (except fabry's and gaucher's)
- Mental retardation in all (except Fabry's and Gaucher's – I)

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Microscopy findings

i. Zebra body inclusion → electron microscopy – common in Niemann pick disease (due to folding of lysosomal membranes)

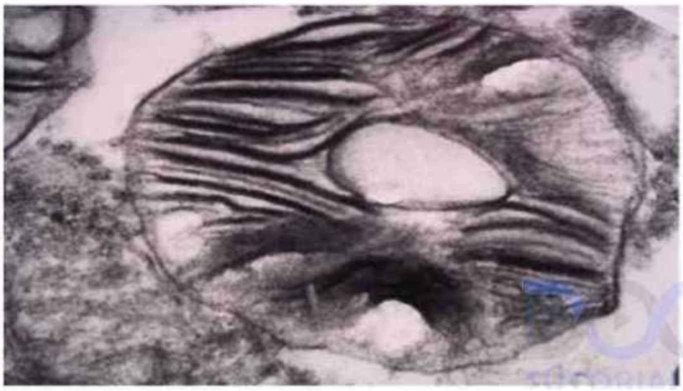

Fig: 1

ii. Globid cell inclusions → light microscopy – common in krabbe's

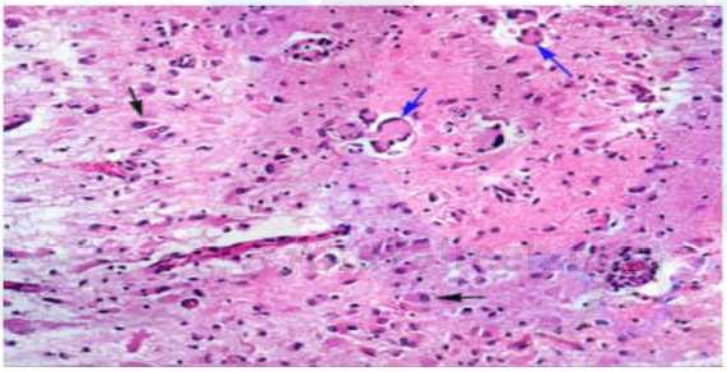

Fig: 2

iii. Gaucher cells (crumpled/wrinkled tissue paper appearance) – Gaucher's disease (characteristic not diagnostic).

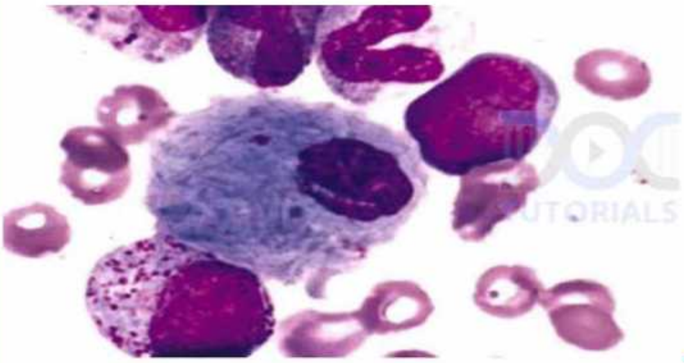

Fig: 3

- Note: Pseudo gaucher cell – seen in CML

Disease	Deficiency of
i. Tay sach's disease	Hexosaminidase A
ii. Sandhoff's disease	Hexosaminidase A and B
iii. Fabry's disease	α – Galactosidase
iv. Metachromatic leukodystrophy	Aryl sulfatase A
v. Krabbe's disease	β – Galactosidase
vi. Gaucher's disease	β – Glucosidase
vii. Niemann pick disease	Sphingomyelinase
viii. Farber disease	Ceramidase

- All disease except niemann pick disease, the defect in breakdown of sphingoglycolipids
- So sphingomyelin accumulates only in neimann pick disease

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Treatment:

- Enzyme replacement therapy (ERT) – repeated frequently
- Bone marrow transplant
  - Good matched donor
  - Lifelong immunosuppression
  - commonly used in Gaucher' and Fabry's
- Substrate deprivation therapy (sphingosine is removed)

- Chemical chaperone therapy – expose some other bond of the molecule for which enzyme is available.

- Gene therapy
  - In vivo (success rate 15-20%) → vector with correct gene copy is infused into patient.
  - Ex vivo (success rate ~ 80%) → extract stem cells by flow cytometry

    ↓

    Introduce correct gene copy inside cell by gene gun/vector/electroporation

    ↓

    re-infuse into patient

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Gaucher's disease

- Most common lysosomal storage disorders
- β – glucosidase/glucocerebrosidase/glycosyl ceramidase deficiency.
- Cherry red spot not seen
- Mental retardation – not seen in type I
- Gaucher's cell on microscopy
- Clinical presentation: abdominal and bone pain
- Biochemical findings:
  - Increased chitotriosidase
  - Increased ACE (Angiotensin Converting Enzyme)
  - Increased TRAP (Taratarate Resistant Acid Phosphastase)

  - Increased CCL-18 or PARC (Pulmonary and Activation – Regulated Chemokine)
- Confirmation of diagnosis by – genetic analysis
- Starting of therapy can be based on enzymatic assay

FATTY ACIDS

- Named after corresponding hydrocarbon
- Present as esters in fats and oils (have even number of carbon atoms)
- Some times as non-esterfied form, known as free fatty acid
  - In plasma-combined with albumin
  - In cell-attached to fatty acid binding protein
- The more appropriate term used to describe the fatty acid (long chain) – Non esterfied fatty acid (NEFA)

Fatty acid

i. Saturated

Depending on size of carbon chain

- Short chain fatty acid (SCFA) – C_2 to C_6
- Medium chain fatty acid (MCFA) – C_8 to C_{12}
- Long chain fatty acid (LCFA) – C_{14} to C_{18}

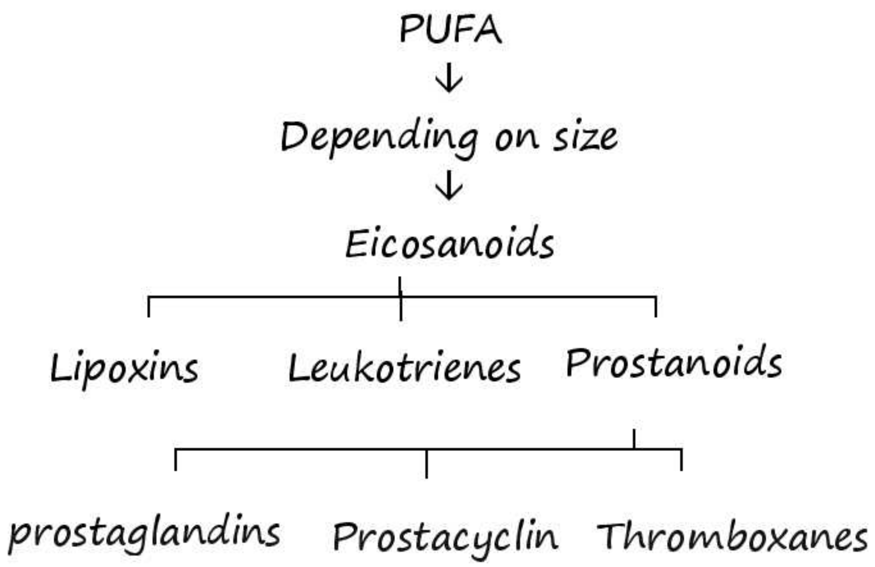
Metabolism in Mitochondria

- Very long chain fatty acid (VLCFA) – $\geq C_{20}$ → metabolism in peroxisome

ii. Unsaturated

[some are essential fatty acid]

- MUFA (single = or = bond)
  [Mono Unsaturated Fatty Acid]
- PUFA (multiple = or = bond)
  [Poly Unsaturated Fatty Acid]


PUFA
Depending on size
Eicosanoids
Lipoxins
Leukotrienes
Prostanoids
prostaglandins
Prostacyclin
Thromboxanes

Essential fatty acids:

- Fatty acid obtained from diet, but not synthesized by body
- Body cannot introduce unsaturated bonds at w-3 and w-6 position
- w-3 series, w-6 series
- w position → position from last carbon

Ex – 18C fatty acid ↗ w-3 – δ 16 position
↘ w – 6 – δ 12 position

w – 3 series → α linolenic acid

i. Eicosa pentaenoic acid (EPA)/Timnodonic acid

ii. Docosa hexaenoic acid (DHA)/cervonic acid

w-6 series → Linoleic acid
↓
Γ – linolenic acid
↓
Arachidonic acid

W-7 series → Oleic acid (synthesized within body)

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- Linoleic acid: most important essential fatty acid safflower oil (richest source) > corn oil 57% > sunflower oil 56% > soyabean oil 51%
- Linolenic acid (α and Γ): soyabean oil
- Arachidonic acid: meat, egg, milk
- EPA: fish
- DHA: Breast milk

Cardioprotective effective of unsaturated fatty acid:

w-3 PUFA > MUFA

(Best cardioprotective effect)

- Diet intake ratio for best effect → PUFA: MUFA = 3:1

FATTY ACID OXIDATION

Types of mechanism of fatty acid oxidation

- α oxidation: peroxisome → metabolism of branched chain fatty acid
- β oxidation: mitochondria → metabolism of SCFA, MCFA, LCFA
- ω oxidation: endoplasmic reticulum → rare
- Peroxisome – also metabolises VLCFA (similar to β-oxidation)

Fatty acid activation

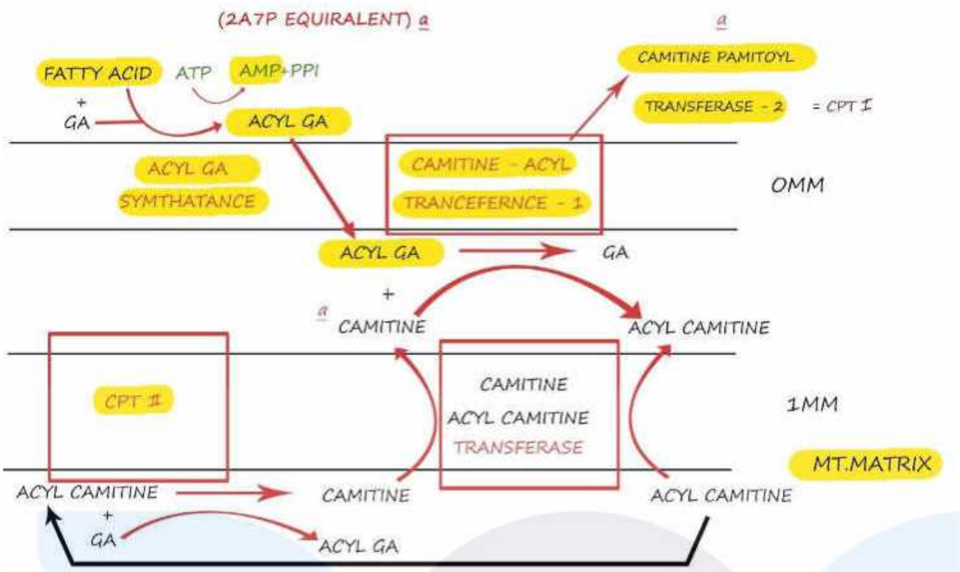
$$\text{Fatty acid} \xrightarrow[\text{Acyl CoA synthetase}]{\text{(Mitochondria, peroxisome, endoplasmic reticulum enzyme)}} \text{Acyl CoA}$$

- SCFA
- MCFA
- LCFA

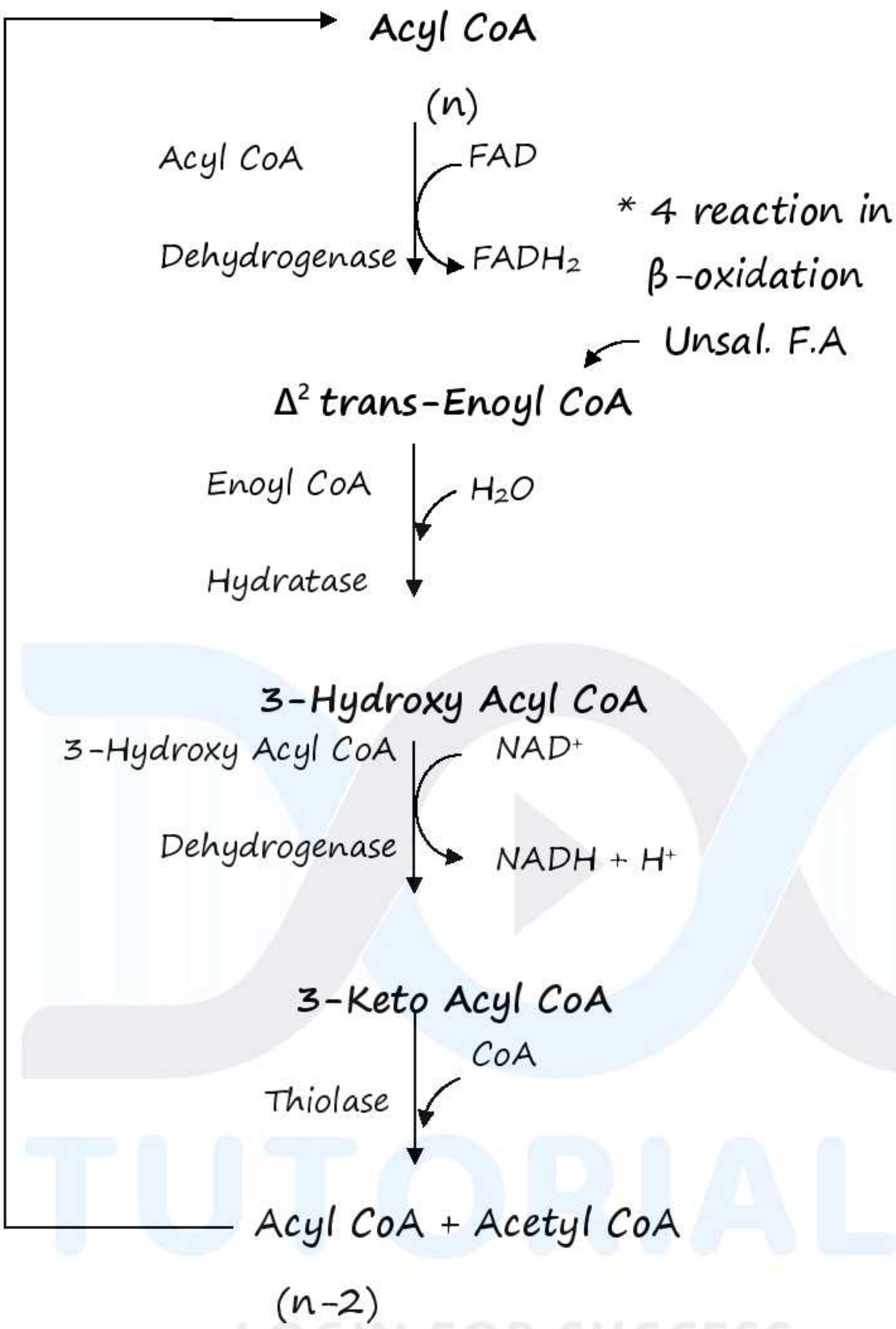
enter mitochondria by carnitine transport system

- SCFA, MCFA → can also enter even without carnitine transport system

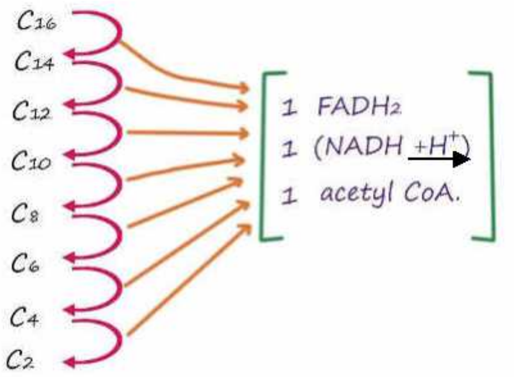
Carnitine transport system


(2A7P EQUIRALENT) a
a
FATTY ACID
ATP
AMP+PPI
+
GA
ACYL GA
CAMITINE PAMITOYL
TRANSFERASE - 2
= CPT I
ACYL GA
SYMTHATANCE
CAMITINE - ACYL
TRANCEFERNCE - 1
OMM
ACYL GA
GA
+
a
CAMITINE
ACYL CAMITINE
CPT II
CAMITINE
ACYL CAMITINE
TRANSFERASE
1MM
MT.MATRIX
ACYL CAMITINE
CAMITINE
ACYL CAMITINE
+
GA
ACYL GA

- Carnitine acyl transferase I/ carnitine palmitoyl transferase I (CPTI)
- CPTI-I → Rate limiting enzyme for β-oxidation
- CPT-I & II → are isoenzymes


Acyl CoA
(n)
Acyl CoA
Dehydrogenase
FAD
FADH₂
* 4 reaction in
β-oxidation
Unsal. F.A
Δ² trans-Enoyl CoA
Enoyl CoA
Hydratase
H₂O
3-Hydroxy Acyl CoA
3-Hydroxy Acyl CoA
Dehydrogenase
NAD⁺
NADH + H⁺
3-Keto Acyl CoA
CoA
Thiolase
Acyl CoA + Acetyl CoA
(n-2)

- Example: Palmitic acid= C_{16} (long chain saturated fatty acid)


C16
C14
C12
C10
C8
C6
C4
C2
1 FADH2
1 (NADH +H⁺)
1 acetyl CoA.

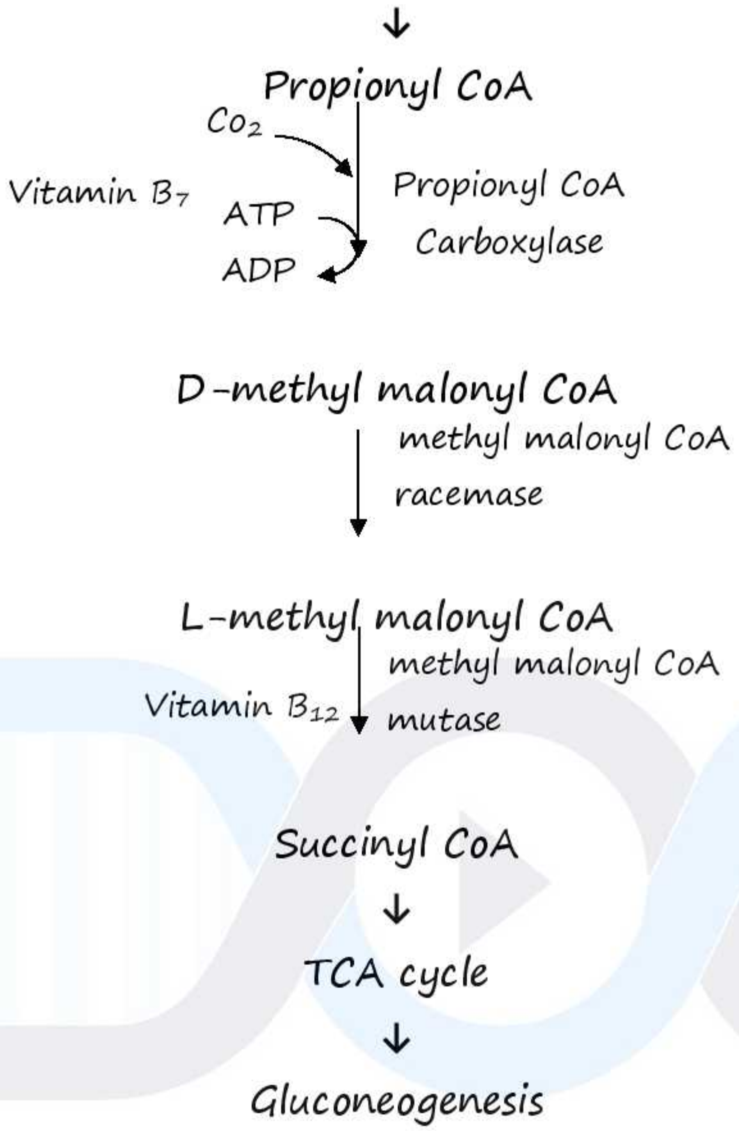
set of products after every round of β-oxidation

- To completely breakdown C_{16} till C_2, the
  - Number of cycle required = $(n/2)-1 \rightarrow (16/2)-1 = 7$
  - Number of $FADH_2$ = $(n/2)-1 \rightarrow (16/2)-1 = 7 \rightarrow 7 \times 1.5 = 10.5$
  - Number of $NADH + H^+ = (n/2)-1 \rightarrow (16/2)-1 \rightarrow 7 \times 2.5 = 17.5$
  - Number of acetyl CoA = $(n/2)-1+1 = (n/2) \rightarrow (16/2) = 8 \rightarrow 8 \times 10 = 80$

Example:

- C_{18} saturated fatty acid (stearic acid)
  - $n=18$
  - $FADH_2 \rightarrow (18/2)-1 = 8 \times 1.5 = 12$
  - $NADH + H^+ \rightarrow (18/2)-1 = 8 \times 2.5 = 20$
  - Acetyl CoA $\rightarrow (18/2) = 9 \times 10 = 90$
  - Total = 122-2 = 120 ATP

- If odd number of carbon (odd chain fatty acid metabolism)
- Example: C_{17}, C_{15}, C_{13}, C_{11}, C_9, C_7, C_5, C_3 (cannot be metabolised by β-oxidation)


Propionyl CoA
CO_2
Vitamin B_7
ATP
ADP
Propionyl CoA Carboxylase
D-methyl malonyl CoA
methyl malonyl CoA racemase
L-methyl malonyl CoA
Vitamin B_{12}
methyl malonyl CoA mutase
Succinyl CoA
TCA cycle
Gluconeogenesis

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- Vitamin B_{12} deficiency → L-methyl malonyl CoA $\not\rightarrow$ Succingl CoA

↓

- Methyl malonyl aciduria
- Used to identify B_{12} deficiency

α-oxidation

- Removal of one carbon at a time from carboxyl end
- Occur in peroxisome
- Target for α-oxidation in humans' diet → phytanic acid from plants branched at β-site. So, β oxidation not possible

- Defect in α-oxidation → Phatanoyl CoA dioxygenase deficiency

↓

Ref sum's disease

↓

No accumulation of phytanic acid in CNS because phytanic acid do not cross blood brain barrier

- Peroxisomes
  - Absent i.e., no synthesis (or) Empty i.e., no enzymes synthesis s] Zellweger's syndrome (or) cerebrohepatorenal syndrome
  - Accumulation of VLCFA in CNS due to defective VLCFA metabolism and accumulation of VLCFA and branched fatty acid (phytanic acid) in rest of body

ω-oxidation

- Oxidation of ω carbon (carbon which is most distant from carboxyl group)
- Occurs in endoplasmic reticulum of liver and kidney cells
- Minor pathway
- Enzymes involved
  - Mixed function oxidase
  - Alcohol dehydrogenase
  - Aldehyde dehydrogenase] also used for alcohol metabolism

FATTY ACID SYNTHESIS

- Lipogenesis – 2 steps → Fatty acid synthesis; → Esterification → lipids
- Fatty acids synthesis → ester → lipids
- For fatty acids from outside the tissue (ex: In adipocytes, fatty acids obtained from circulation i.e., from liver) → esterification → lipids
- Location where fatty acids synthesis upregulated
  - Liver
  - Kidney
  - brain
  - Lungs
  - Lactating mammary gland
  - Adipocytes
- Compartment: cytoplasm
- Substrate: Acetyl CoA (of carbohydrates origin)

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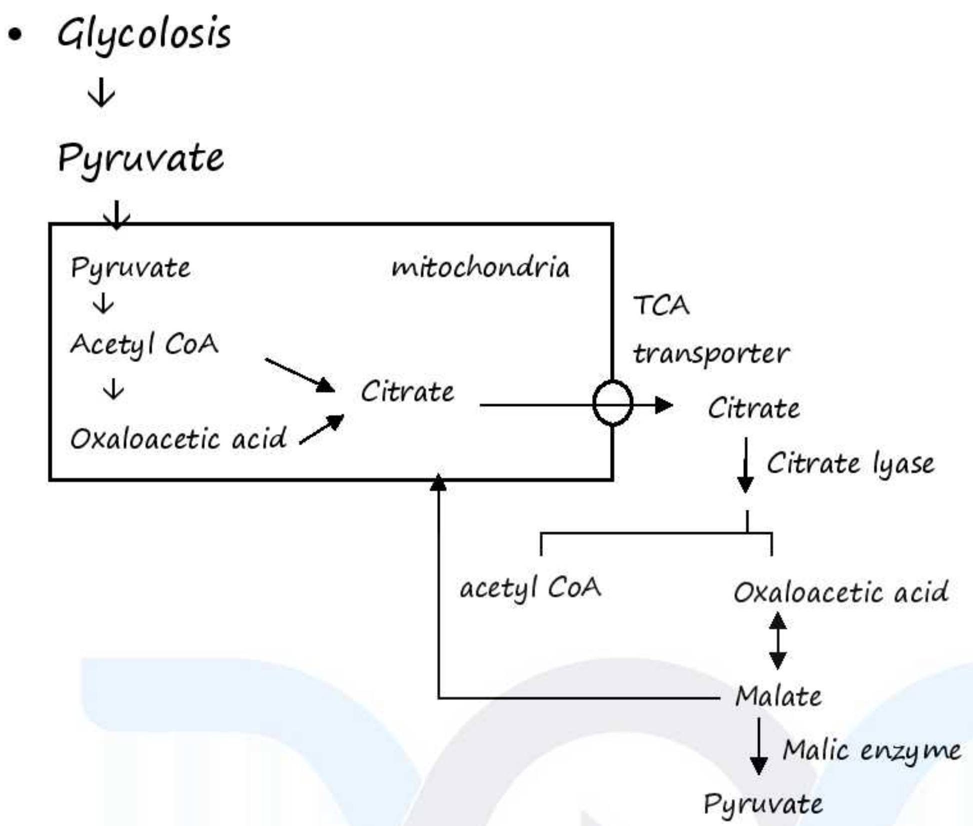
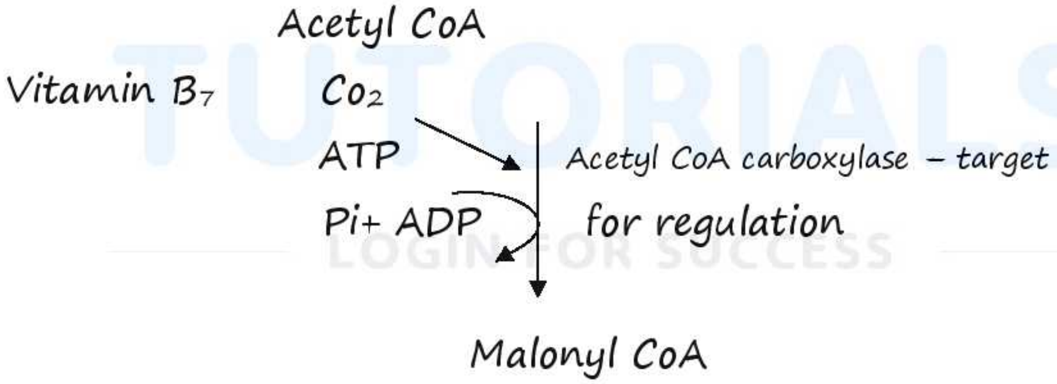

• Glycolysis
Pyruvate
Pyruvate
mitochondria
Acetyl CoA
Oxaloacetic acid
Citrate
TCA
transporter
Citrate
Citrate lyase
acetyl CoA
Oxaloacetic acid
Malate
Malic enzyme
Pyruvate

- TCA transporter (low sensitivity i.e., acts only when concentration of citrate is high in mitochondria)


Acetyl CoA
Vitamin B7
Co2
ATP
Pi+ ADP
Acetyl CoA carboxylase – target
for regulation
Malonyl CoA

- Components of Acetyl CoA carboxylase:
  - – Biotin
  - – 2 enzymes
    - Biotin carboxylase
    - Carboxyl transferase
  - – Biotin carboxyl carrier protein

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- Reaction:

  CO_2 + Acetyl CoA
  ↓
  Biotin – Coo + acetyl CoA
  ↓ arm action of biotin
  Biotin + Malonyl CoA

- Acetyl CoA carboxylase usually exist in polymeric forms

  Acetyl CoA
  ↓
  Malonyl CoA

Fatty acid synthase complex components:

i. Malonyl/Acetyl transacylase
ii. Acyl carrier protein
iii. Keto acyl synthase
iv. Keto acyl reductase
v. Dehydratase
vi. Enoyl reductase
vii. Thio esterase

E = cysteine – Acetyl C_2
N ↓ Malonyl/Acetyl transacylase
Z = Panthotenic acid – Malonyl C_3
CO_2 ← ↓ Ketoacyl synthase

E = cysteine
N
Z = Panthotenic – Ketoacyl (C_4)

$$\begin{tikzcd}\text{NADPH} + \text{H}^+ \arrow[d, bend left] & \arrow[d, "\text{Keto Acyl Reductase (Reduction)}"] \\ \text{NADP}^+ & {}\end{tikzcd}$$

↓ Dehydratase (Dehydration)

$$\begin{tikzcd}\text{NADPH} + \text{H}^+ \arrow[d, bend left] & \arrow[d, "\text{Enoyl Reductase (reduction)}"] \\ \text{NADP}^+ & {}\end{tikzcd}$$

E = cysteine

N

Z = Panthotenic acid – Acyl group (C_4)

↓

E = Cysteine – Acyl group

N

Z = Panthotenic acid – Malonyl CoA

↓

Stop at C_{16} (Palmitic acid)

↓ Elongation

In Endoplasmic reticulum [In CNS, VLCFA – C_{22}, C_{24}]

(Fatty acid elongation system)

- The primary source of NADPH in fatty acid synthesis is HMP shunt (85% of NADPH inside cell from HMP shunt)
- End product – variable
- Most common end product – palmitate
- In lactating breast – most common end product + MCFA (C_8 C_{10} C_{12})
- Fatty acid synthase complex is functional only as a dimer

Regulation of acetyl CoA carboxylase/Fatty acid synthesis:

- Allosteric regulation
  - ⊕ Citrate
  - ⊖ End product (long chain acyl CoA)
- Hormonal regulation
  - Phosphorylation (Glucagon/Epinephrine)
  - Dephosphorylation (Insulin)
- Acetyl CoA carboxylase upregulated when excess glucose is there

Phosphorylated from gets inactivated

Insulin activate dephosphorylated form

Increases genetic expression of
- Acetyl CoA carboxylase
- Fatty acid synthase complex

Other effects
- Increases glucose transport into cell
- Increases pyruvate formation
- Increases glycerol 3 phosphate formation

↓

Esterfication

↓

TAG

- Decreases / - lipolysis

Cofactors requires for fatty acid synthesis:

- $NADPH^+$ H^+
- ATP
- Mn^{+2}
- B_7/Biotin
- HCO_3^-

- At what position will you find the carbon of acetyl CoA in final product,

  A: By number, last 2 carbon

  ex: Palmitic acid (C_{15}, C_{16})

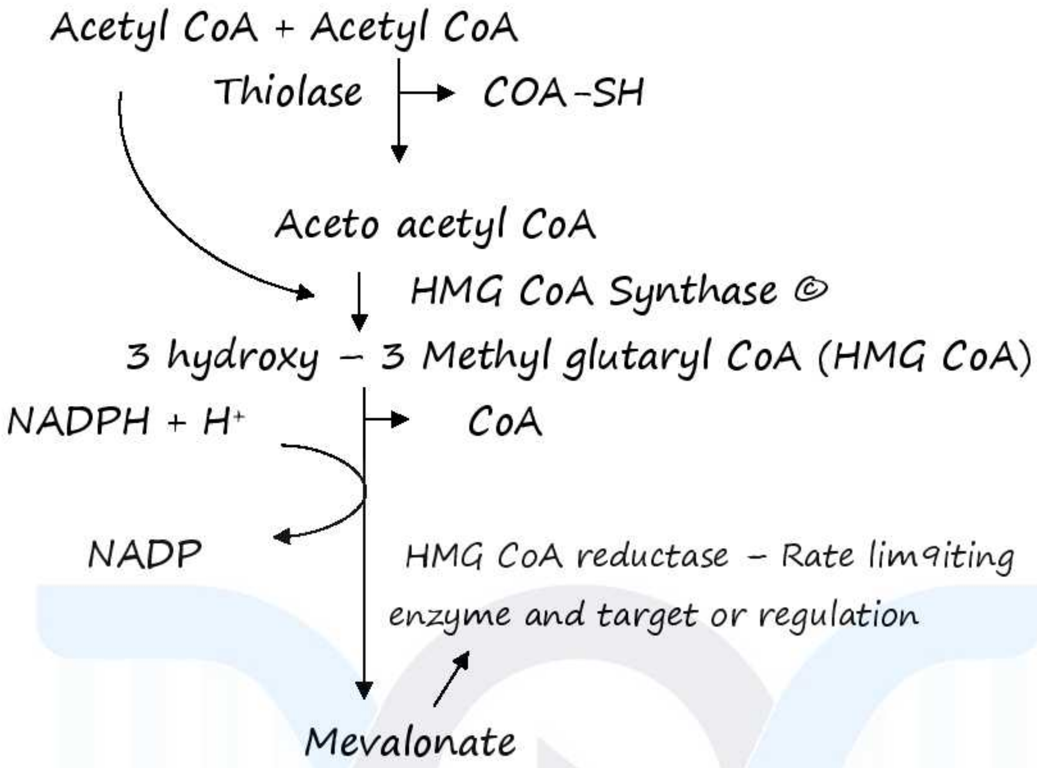
CHOLESTEROL SYNTHESIS

- Cholesterol → Found only in animals not in plants (Ergosterol)
- Cholesterol acts as precursor for various molecules
  - Corticosteroids
  - Sex hormones
  - Bile acids
  - Vitamin D3
- It increases flexibility of cell membrane.
- Effect of cholesterol on membrane:
- More is the content of cholesterol lesser will be the fluidity at high temperature – because of large size of cholesterol
- ↑ Fluidity at low temperature – Irregular size
- If ↑ Cholesterol – Gradual transition on high temperature from semi – rigid to fluid state of membrane and vice versa

Synthesis

- By all tissues, maximum synthesis by liver (10%) and intestines (10%)
- 50% from diet 50% synthesis in body
- Dietary and synthesis component will be in equilibrium
- Donor for all carbon atoms of carbon – Acetyl CoA
- Stage 1: Mevalonate synthesis
- Stage 2: Isoprenoid units
- Stage 3: 6 Isoprenoid units form squalene
- Stage 4: Lanosterol synthesis
- Stage 5: Cholesterol

Stage: 1


Acetyl CoA + Acetyl CoA
Thiolase
COA-SH
Aceto acetyl CoA
HMG CoA Synthase ©
3 hydroxy – 3 Methyl glutaryl CoA (HMG CoA)
NADPH + H+
CoA
NADP
HMG CoA reductase – Rate lim9iting enzyme and target or regulation
Mevalonate

HMG CoA Reductase:

- Feed back inhibition by mevalonate
- Feed back inhibition by bile acids
- Feedback inhibition by cholesterol

acting through SREBP (Sterol) Regulatory element binding

↓

Act at genetic level

↓

Prevent transcription of HMG CoA reductase

- Statins inhibits synthesis of HMG CoA reductase
- Hormonal regulation by insulin and thyroid – ↑ HMG CoA reductase activity
- Glucagon/Glucocorticoid – ↓ activity
- Excess cholesterol promotes degradation of HMG CoA reductase

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Mevalonate

↓ 3 kinase decarboxylases

Isopentenyl Di-Phosphate

↓ 6 Isoprenoid units are used up

Squalene

↓ Squalene epoxidase

Lanosterol

Δ24 reductase

Cholesterol

- Statins – Immediate ↓ in amount of cholesterol synthesis

  ↓

  Loss of feedback by cholesterol at SREBP

  ↓

  So, effect of statins gradually diminishes

KETOGENESIS

Ketone bodies

- Acetone – Volatile → Fruity odour of urine/breath in case of ketosis
- Acetoacetate – 1st ketone body to be synthesized
- β hydroxybutyrate=pseudo ketone body because of no keto group

Synthesis:

- Adipocyte

TAG
↓ Hormone Sensitive Lipase ← (–) Insulin
Glycerol + Free fatty acid

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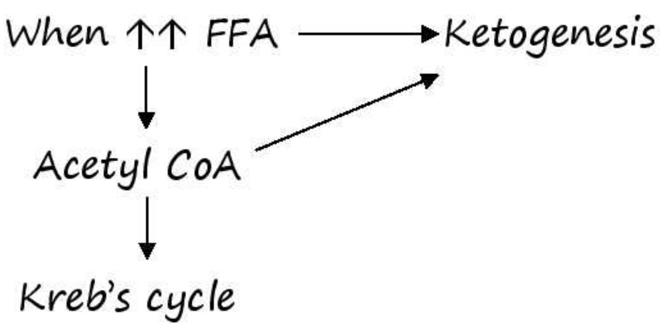
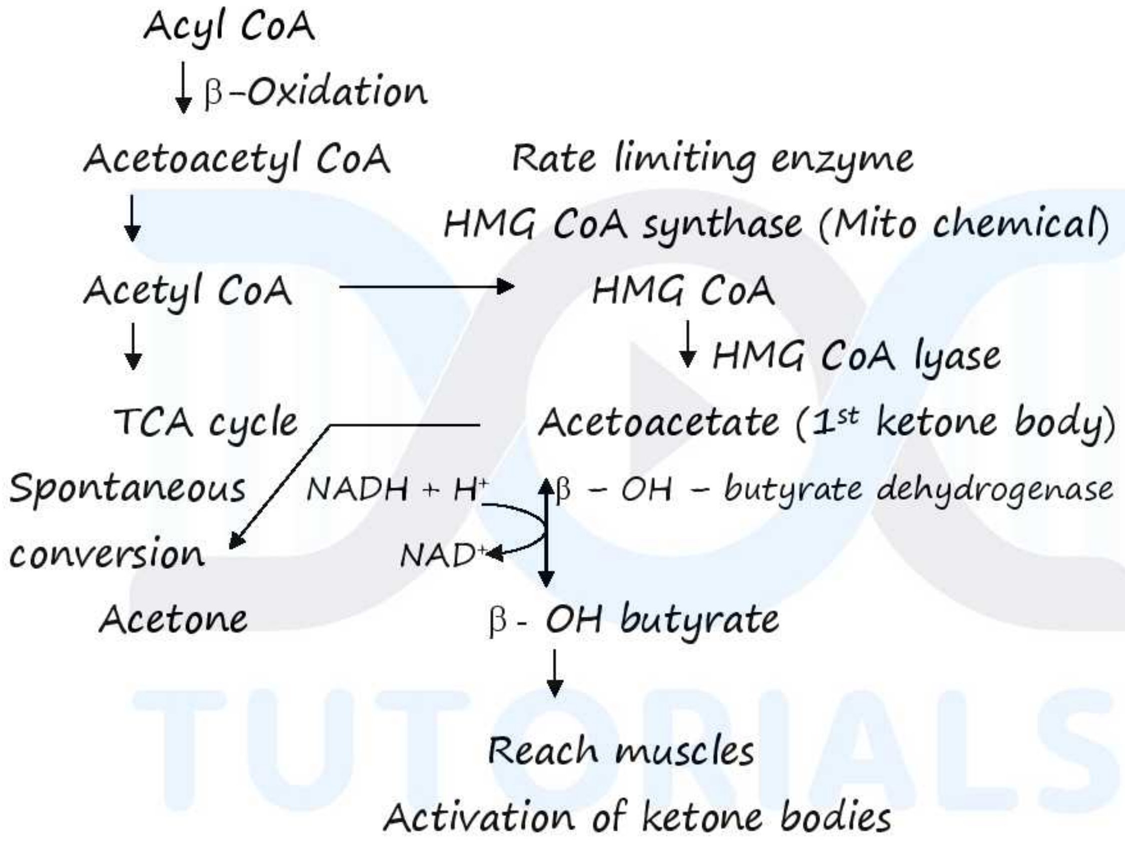
Fasting state:

Insulin ↓
↓ No (–)
Hormone sensitive lipase
Triacylycerol → Glycerol + free fatty acid (FFA)
↓
Blood
↓
Liver

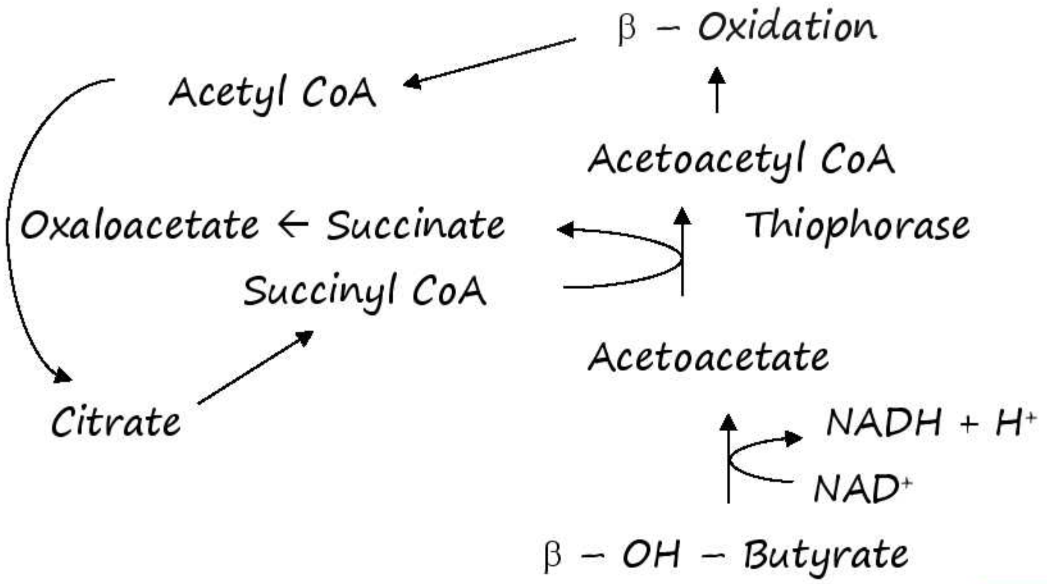
- Amount of energy from fatty acid metabolism in liver has a ceiling because of limited amount of NAD in hepatocytes


When ↑↑ FFA
Ketogenesis
Acetyl CoA
Kreb's cycle

In liver


Acyl CoA
β–Oxidation
Acetoacetyl CoA
Rate limiting enzyme
HMG CoA synthase (Mito chemical)
Acetyl CoA
HMG CoA
HMG CoA lyase
TCA cycle
Acetoacetate (1st ketone body)
Spontaneous
conversion
NADH + H+
β – OH – butyrate dehydrogenase
NAD+
Acetone
β - OH butyrate
Reach muscles
Activation of ketone bodies

Circulation:


β – Oxidation
Acetyl CoA
Acetoacetyl CoA
Oxaloacetate ← Succinate
Thiophorase
Succinyl CoA
Acetoacetate
Citrate
NADH + H+
NAD+
β – OH – Butyrate

- In liver – no thiphorase – so liver cannot metabolize ketone bodies
- Thiophorase also known as succinyl CoA acetoacetate transferase
- In type II Diabetes – Hyperinsulinemia

  ↓

  non ketotic hyperosmolar coma
- In type I Diabetes – no insulin

  ↓

  Diabetic keto acidosis (DKA)

Conditions in which significant ketogenesis occur:

- DKA
- Starvation
- Chronic alcohol intoxication
- Ketogenesis

  ↓

  Ketosis → Ketonuria → Nitroprusside based test (Rotheras nitroprusside test)

  Ketosis ↓ Ketoacidosis

  Ketonuria → FeCl3 based test (Gerhardt's test)
- Both the above tests are based on presence of keto group i.e. both are negative for β-OH butyrate

BILE ACID SYNTHESIS

Functions of Bile acids

- Emulsification of fats in intestine for absorption
- Emulsification – breaking of big lipid molecules into smaller molecules

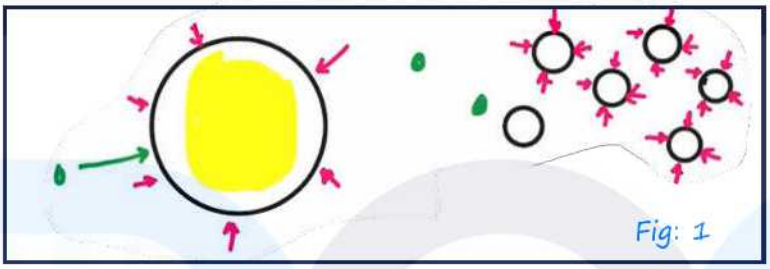


Fig: 1

- In obstructive jaundice, bile acids are not released into intestine

↓

Impaired fat absorption

Steatorrhea

↓lipid soluble vitamin absorption

↓

Vitamin K deficiency

– Bleeding tendency

Bile acid synthesis

- Only in liver
- Secreted in intestine, reabsorbed and enters liver → Enterohepatic circulation (98–99% reabsorbed in ileum)
- Compartment: Endoplasmic reticulum (microsome), cytoplasm, peroxisome.

LIPOPROTEINS

- Lipoproteins are specialized structures used for transport of lipids as lipids cannot travel freely in blood due to lipophilic nature
- Well defined structure – Outer and inner layer
- Outer layer – Amphipathic molecule (Hydrophilic at one end and hydrophobic at another end)

  i. Phospholipids
    - PO4 interact with blood
    - Lipid interact with non-polar core

  ii. Cholesterol
    - Multiple OH group interact with blood
    - Ring structure interact with non-polar core

  iii. Apolipoproteins
- Non – Polar core
  - TAGS/TGS (triglycerides)
  - Cholesteryl esters

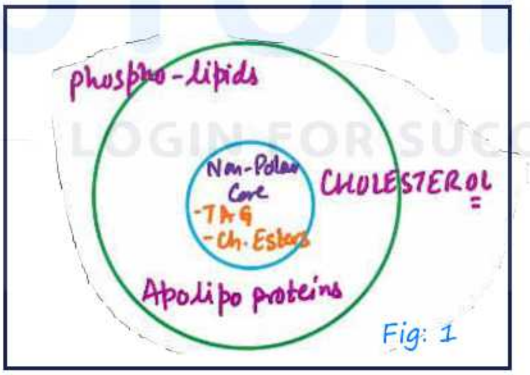

phospho-lipids
Non-Polar Core
-TAG
-Ch. Esters
CHOLESTEROL
Apolipo proteins

Fig: 1

Lipoproteins

- Chylomicrons
- VLDL
- LDL
- HDL

Ultracentrifugation:

- Sample in a tube
- Centrifugation
- Separation based on density

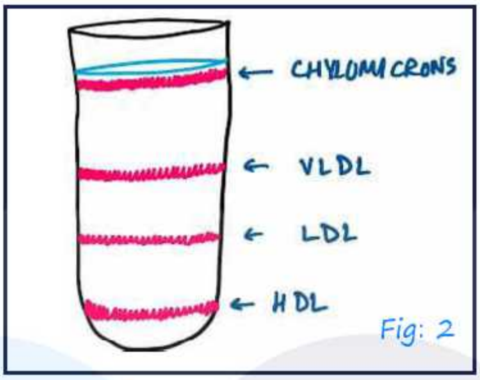

CHYLOMICRONS
VLDL
LDL
HDL

Fig: 2

Electrophoresis:

- Based on charge which is a function of relative protein content

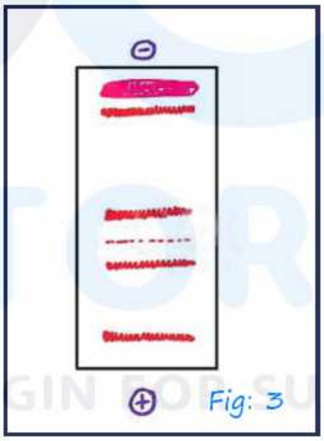


Fig: 3

DOC TUTORIALS
LOGIN FOR SUCCESS

Types:

Name	Source	Function/Content	Marker (Apolipoprotein) and receptors
Chylomicrons	Intestine	Exogenous dietary TAG	B-48 → incomplete protein no receptor
Chylomicron remnant (Chylomicron after delivery its TAG)	Chylomicron	TAG, Cholesterol	B-48, APOE→ LDL receptor, LRP (LDL receptor related protein)
VLDL	Liver	Endogenous/Hepatic TAG	B-100 → LDL receptor C-I,II,III → No receptor (Variants of APOC)
VLDL remnant/IDL (Once VLDL delivered majority of TAG)	VLDL	TAG, cholesterol	B-100, APOE
LDL	VLDL	Cholesterol transport from liver to extrahepatic tissue	B-100
HDL	Liver and intestine	Reverse cholesterol transport	A-I → HDL receptor

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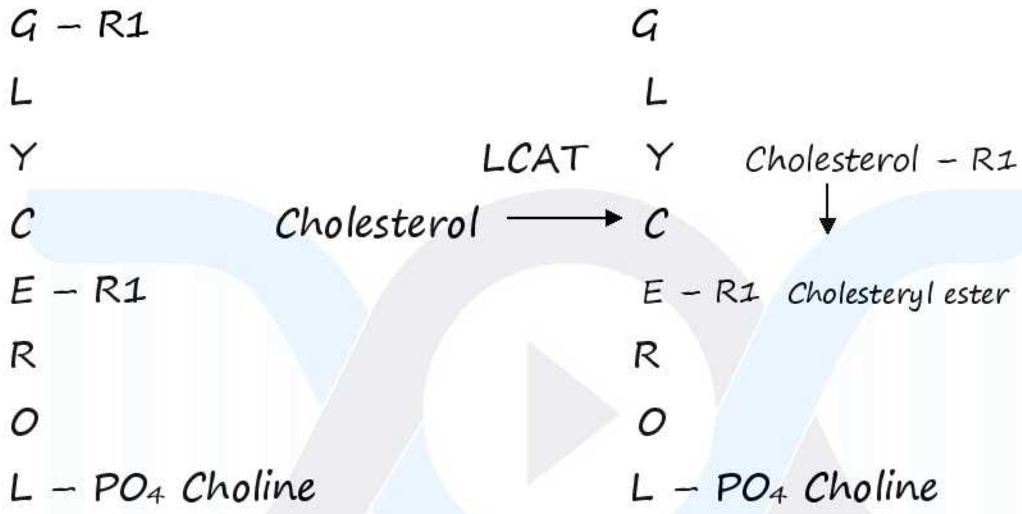
Function of Apolipoproteins

i. Enzyme activators/Cofactors

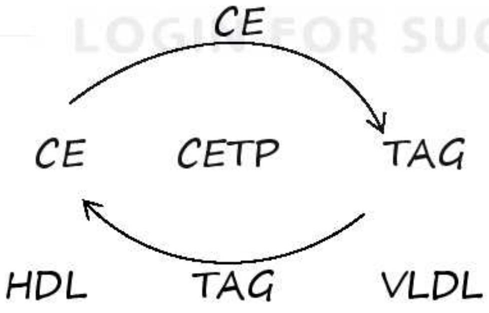
- APO C-II activator for lipoprotein lipase
- APO A-I activator for LCAT (Lecithin Cholesterol Acyl Transferase)

Lecithin


G – R1
L
Y
C
E – R1
R
O
L – PO₄ Choline
Cholesterol
LCAT
G
L
Y
C
E – R1
R
O
L – PO₄ Choline
Cholesterol – R1
Cholesteryl ester

ii. Enzyme inhibitors:

- C III and A II → Inhibit lipoprotein lipase
- C I → Inhibit CETP (Cholesteryl ester transfer protein)


CE
CE
CETP
TAG
TAG
HDL
VLDL

DOC TUTORIALS
LOGIC FOR SUCCESS

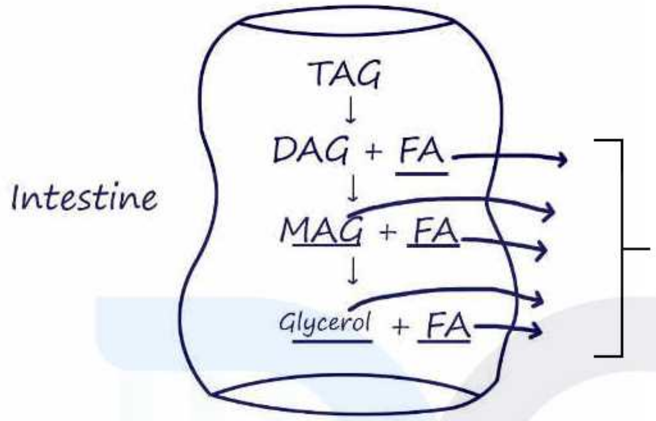
iii. Ligands for receptors:

- APOB – 100 and APOE → LDL receptor
- APOE → LRP
- APO AI → HDL receptor.

LIPOPROTEIN TRANSPORT

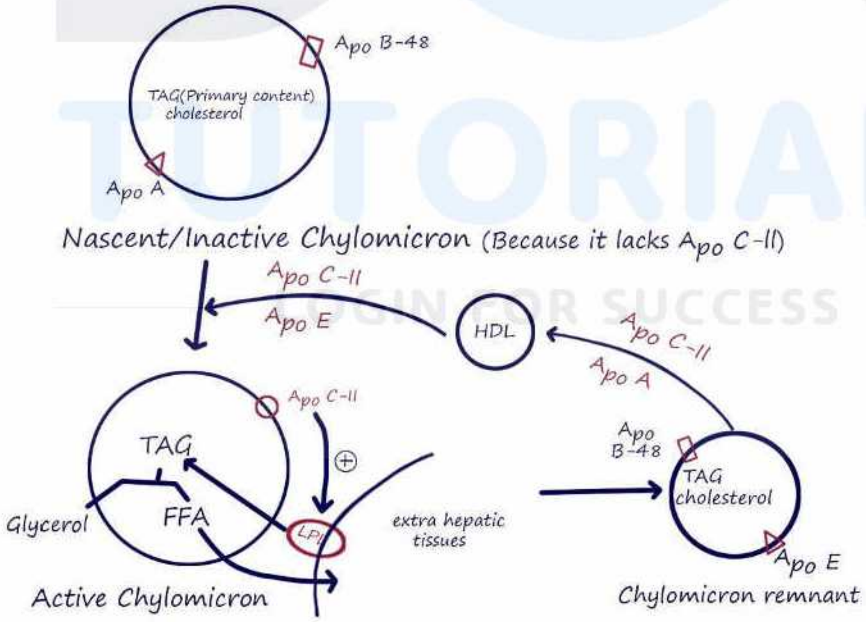
Chylomicron 00:00:27

- Dietary triglycerides/ tri acyl glycerol


Intestine
TAG
DAG + FA
MAG + FA
Glycerol + FA

Reforms as TAG
↓
Packed as chylomicron
↓
Lymphatics
↓
Inferior vena cava
↓
Heart
↓
Rest of the body

Structure


Apo B-48
TAG(Primary content)
cholesterol
Apo A
Nascent/Inactive Chylomicron (Because it lacks Apo C-II)
Apo C-II
Apo E
HDL
Apo C-II
Apo A
Apo C-II
⊕
TAG
Glycerol
FFA
LPL
extra hepatic tissues
Apo B-48
TAG cholesterol
Apo E
Active Chylomicron
Chylomicron remnant

- When the active chylomicron reach extra hepatic tissue

↓

Apo C-II acts on lipoprotein lipase which is attached to capillary endothelium

↓

Lipoprotein lipase acts on TAG

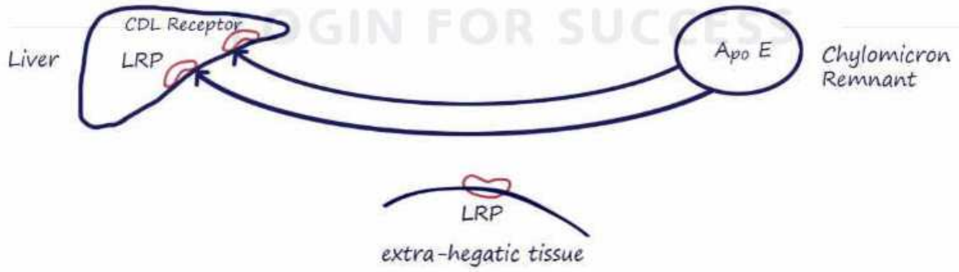
↓

TAG

Glycerol → Liver

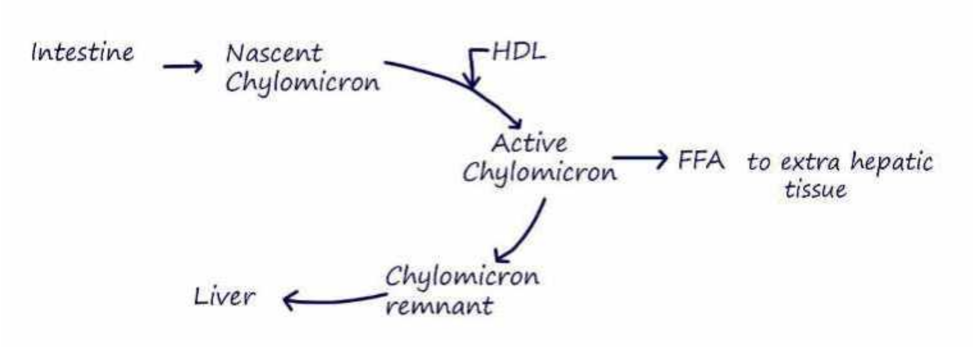
Free fatty acids → Taken up by tissue

- After TAG is delivered, chylomicron becomes small, the surface area decrease and sheds apolipoproteins and becomes chylomicron remnant
- Apo E can be identified by two receptors
  - i. LDL receptor
  - ii. LRP

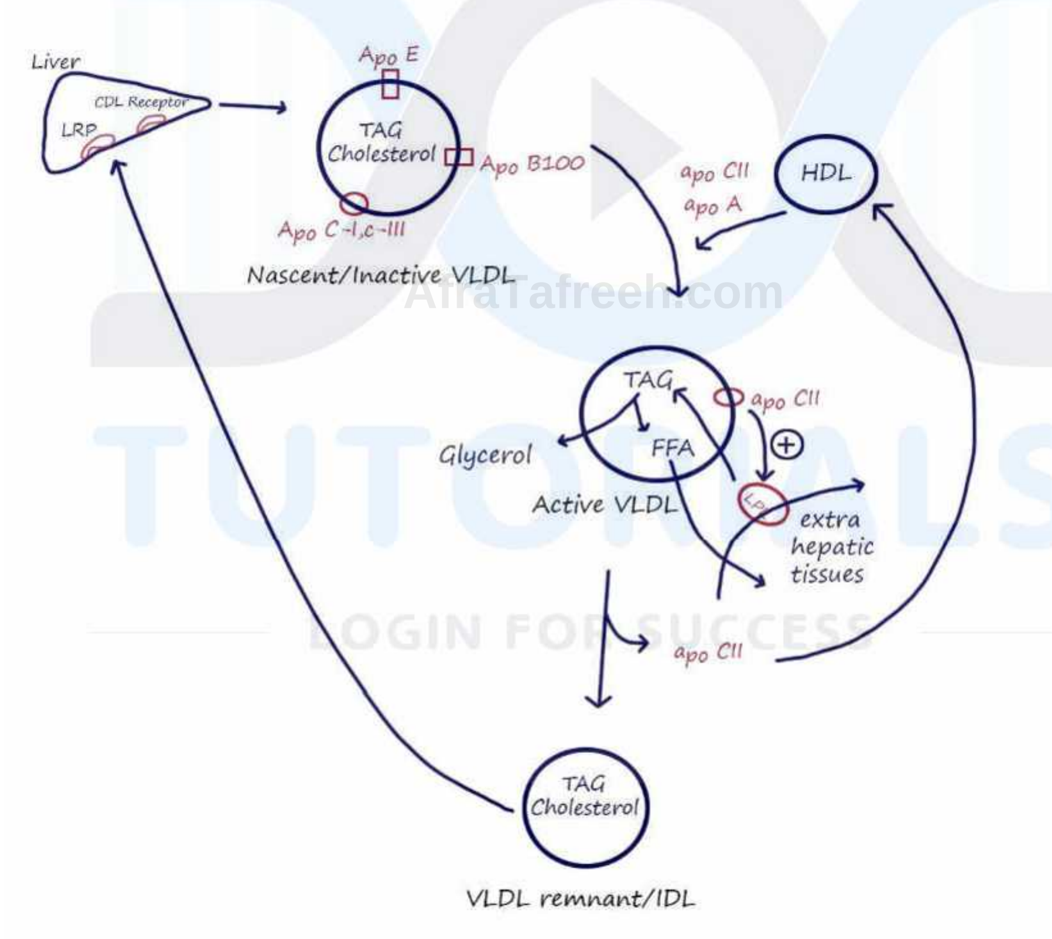

Liver
CDL Receptor
LRP
Apo E
Chylomicron Remnant
LRP
extra-hegatic tissue

- Chylomicron remnant
  - Cannot be taken up by extra-hepatic tissue due to no membrane flexibility
  - Taken up by hepatocytes through sinusoids which have flexible membrane

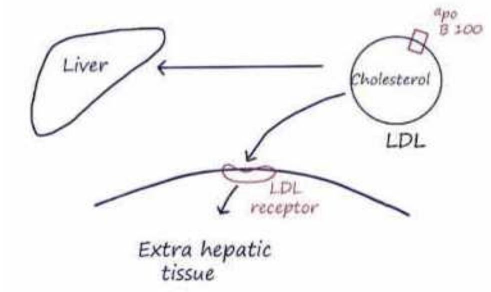
Summary


Intestine
Nascent Chylomicron
HDL
Active Chylomicron
FFA
to extra hepatic tissue
Chylomicron remnant
Liver

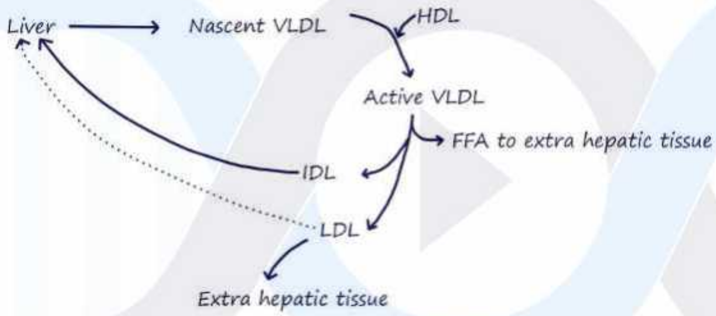
VLDL 00:12:26


Liver
CDL Receptor
LRP
Apo E
TAG
Cholesterol
Apo B100
Apo C-I,c-III
Nascent/Inactive VLDL
apo CII
apo A
HDL
TAG
FFA
Glycerol
apo CII
⊕
LPL
Active VLDL
extra hepatic tissues
apo CII
TAG
Cholesterol
VLDL remnant/IDL

- Sometimes VLDL looses almost all TAG and becomes LDL


Liver
apo
B 100
Cholesterol
LDL
LDL
receptor
Extra hepatic
tissue

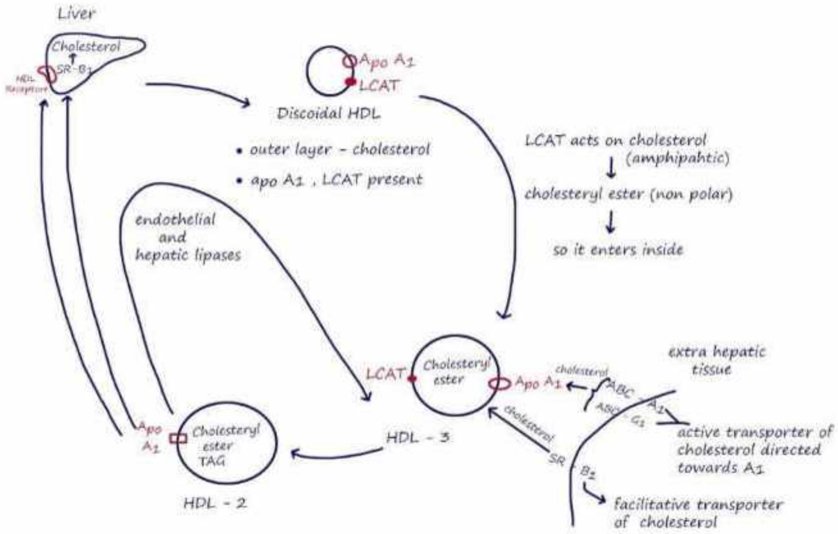
Summary


Liver
Nascent VLDL
HDL
Active VLDL
FFA to extra hepatic tissue
IDL
LDL
Extra hepatic tissue

HDL 00:20:50

- Synthesis
  - Liver
  - Intestine

TUTORIALS LOGIN FOR SUCCESS


Liver
Cholesterol
SR-B1
HDL Receptor
Apo A1
LCAT
Discoidal HDL
• outer layer - cholesterol
• apo A1 , LCAT present
LCAT acts on cholesterol
(amphipahtic)
cholesteryl ester (non polar)
so it enters inside
endothelial
and
hepatic lipases
LCAT
Cholesteryl
ester
Apo A1
cholesterol
ABC - A1
ABC - G1
extra hepatic
tissue
active transporter of
cholesterol directed
towards A1
cholesterol
SR - B1
facilitative transporter
of cholesterol
HDL - 3
Apo
A1
Cholesteryl
ester
TAG
HDL - 2

- Lipase act on cholesteryl ester and release some amount of cholesterol which can be taken up by SRBI and promotes conversion of HDL-2 to HDL-3
- Sometimes Apo A1 gets dislodged
  - Metabolized and excreted by kidney
  - Can cluster with some amount of phospholipids and cholesterol

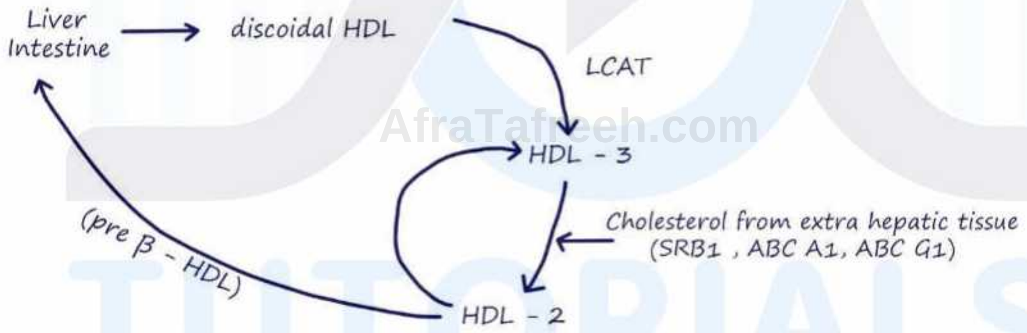
↓

Pre-β HDL

↓

Converted into discoidal HDL

Summary


Liver
Intestine
discoidal HDL
LCAT
HDL - 3
Cholesterol from extra hepatic tissue
(SRB1 , ABC A1, ABC G1)
HDL - 2
(pre β - HDL)

DOC TUTORIALS
LOGIN FOR SUCCESS

21st AND 22nd AMINO ACIDS

21ST Amino Acid

- Selenocysteine (Sec)
- Arises by co-translational insertion of a modified tRNA
- tRNA-serine $\xrightarrow{\text{modified}}$ tRNA – selenocysteine
- Present at active site of many enzymes (>50 enzymes)

  Ex: G6PD

  Glutathione peroxidase
  5^1 – deiodinase
  Thio Redoxin Reductase

  anti-oxidant enzymes
  &
  anti-oxidative function

  Selenocysteine synthase

Note:

- Selenium is not anti-oxidant, but it participates in anti-oxidative function by being present at active site of many enzymes.

Deficiency of selenium:

- Keshan disease
- Kashin Beck's disease

Structure:

- Resembles the structure of cysteine (in place of sulphur → selenium)

Cysteine → sulphur

Selenocysteine → selenium

Note:

- 21st amino acid coded by UGA (stop codon) followed by SECIS (SEC insertion sequence) element

22nd aminoacid: Pyrrolysine

- Found in some methanogenic bacteria, archaebacteria
- Pyrrolysine – Present only in enzyme of methane producing pathway
- Structure is similar to lysine with an extra pyrroline ring
- Coded by UAG codon.

SPECIAL PRODUCTS OF AMINO ACID

Gluconeogenesis

- Amino acids which give rise to glucose are involved in pathway of gluconeogenesis
- All amino acids are involved in gluconeogenesis except lysine & Leucine
- Alanine is the most important or most common to be involved in glucose production

Arginine

- Help in producing

  Creatine (Store house of Po4 in muscle)

  ↓

  Creatine phosphate

  ↓ Spontaneous degradation

  Creatinine

  help in nitric oxide synthesis by using enzyme nitric oxide synthase (Helps in vasodilation)

- Aspartate – Important in production of purine & pyrimidine

Cysteine

- Participate in synthesis of coenzymes A

↓

It also requires Vit B5 for its synthesis

Contributes to synthesis of taurine

Glycine

- Participate in synthesis of creatine.
- contributes atoms to synthesis of purine ring
- Required for heme synthesis
- Help in formation of water-soluble conjugates from insoluble forms
- i.e. when glycine is attached to some insoluble forms it helps in solubility of that compound

Histidine

- An only amino acid to act as butter at physiological PH
- by decarboxylation reaction it produces histamine
- Give risk to carnosine → Homo carnosine
- Gives risk to anserine

Methionine

- Gives rise to S-Adenosyl methionine (Carbon transporter)

↓ ↓

Part from nucleotide   Aminoacid

- Involved in synthesis of creatine

↓

Creatine phosphate

↓ Converted to

Creatinine

- Involved in synthesis of spermidine

↓ Converted to

Putresine

Ornithine (It can give rise to) → Putresine

- Because is also produced from ornithine
- Methionine involved in synthesis of spermine

Tryptophan

- One of the very important precursor amino acids
- gives rise is to serotonin

↓

5 OH Tryptamine (5HT)

↓

5 hydroxy indole acetic acid

- Because presence of 5 HIAA in urine indicates carcinoid tumors because serotonin is excessively produced in this type of tumors
- Gives rise to melatonin (regulates circadian rhythm)
- gives rise to Niacin (B3) Q
- 60Mg tryptophan $\xrightarrow{\text{gives rise}}$ 1mg B3

Tyrosine

- Gives rise to catecholamines

Dopamine
↓
Norepinephrine
↓
Epinephrine

Tyrosine gets converted into thyroxine hormones (T4 or T3)

Gives rise to melanin (In melanocytes) (By use of tyrosinase)

Glutamate

- Undergoes decarboxylation to give rise to GABA (γ amino butyric acid)

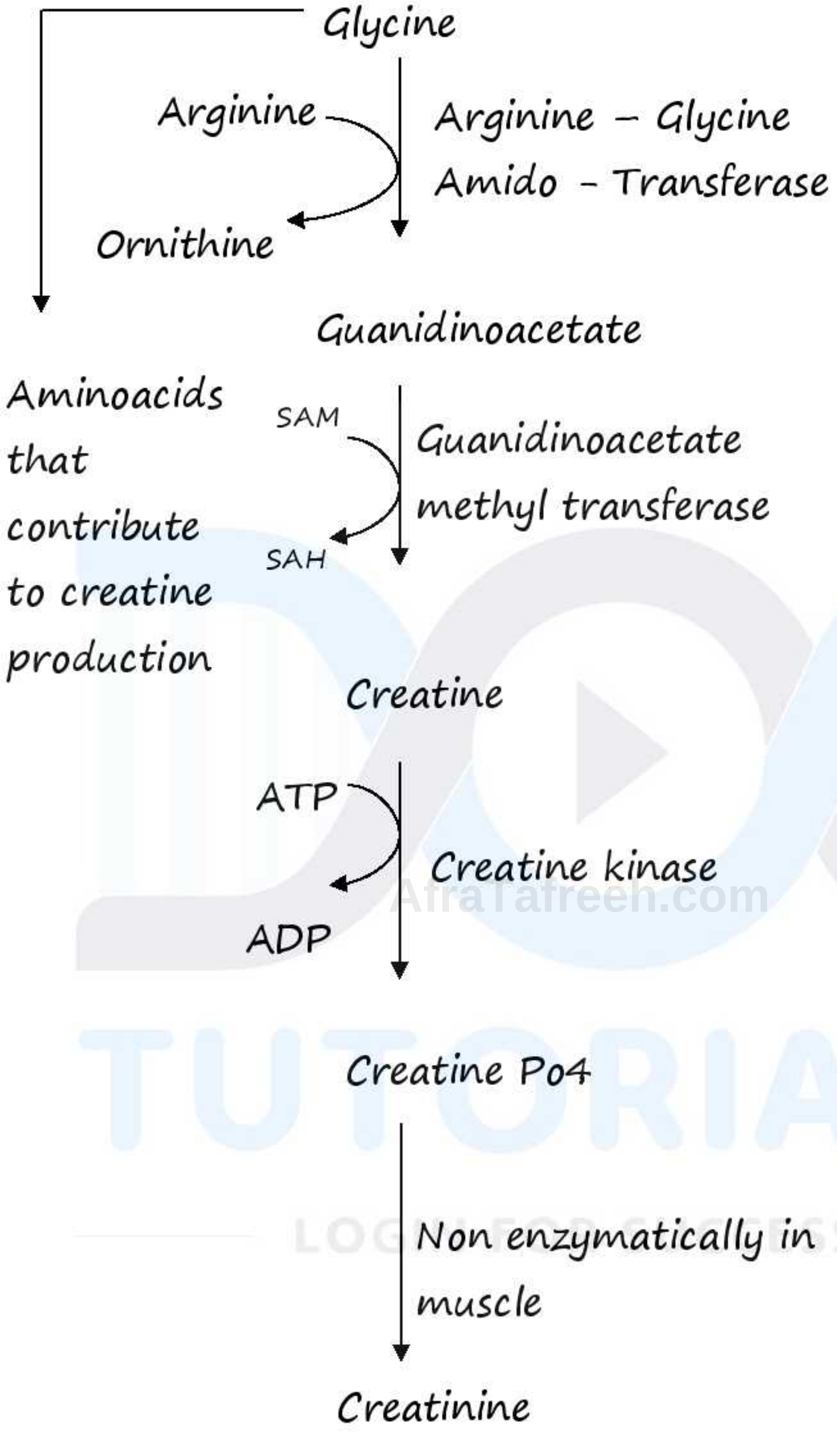
Glutamine

- Synthesis of purine & pyrimidine ring it contributes amide nitrogen in synthesis of purine & pyrimidine ring

Targets of phosphorylation 00:16:21

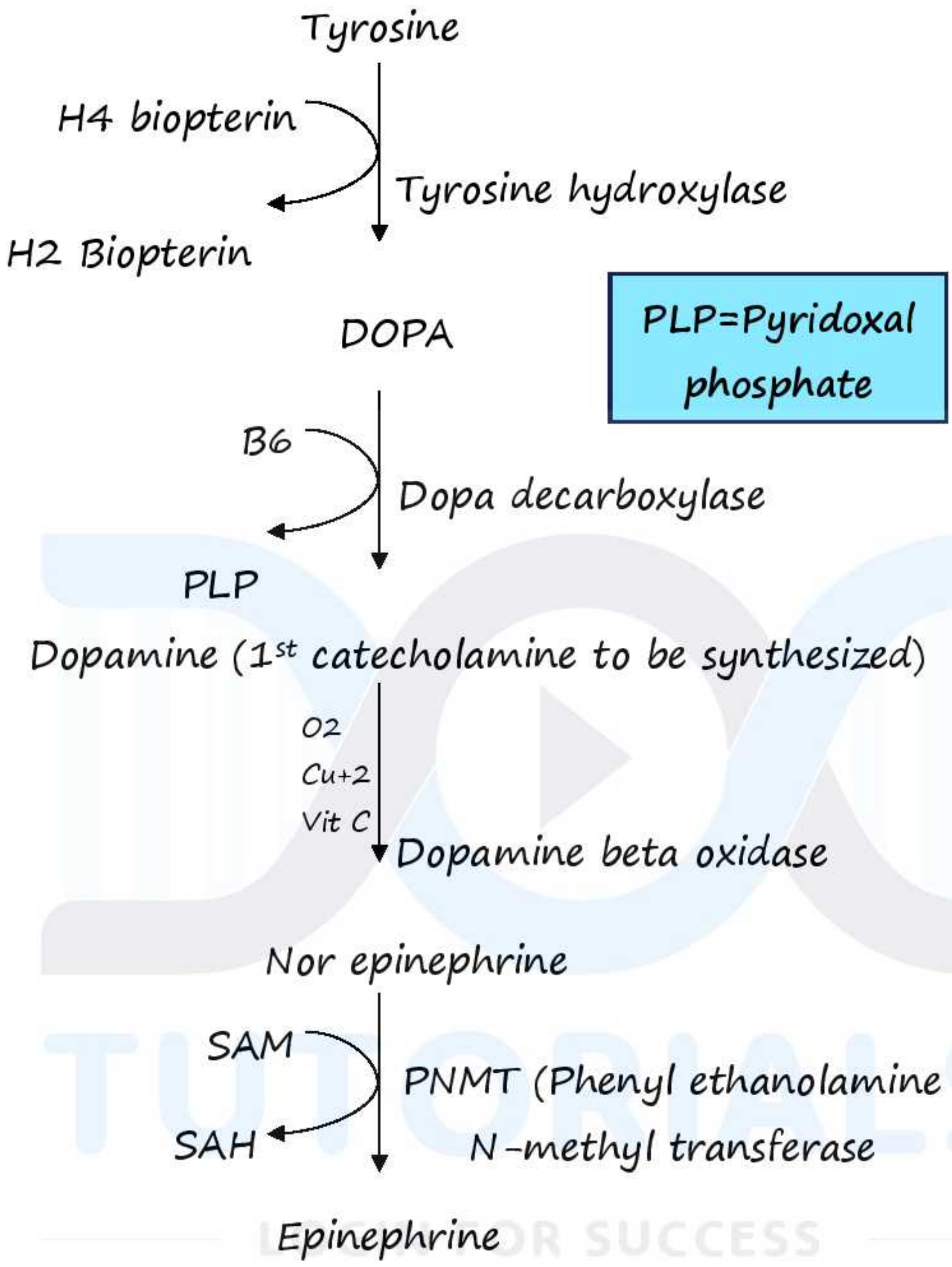
- These are targets for phosphorylation because of presence of hydroxyl groups in side chain so it is able to interact with phosphate group and undergo phosphorylation
- These are also targets for glycosylation but tyrosine is rarely used (Because tyrosine is used only in glycogen synthesis
- Serine, threonine → Comes under O-Glycosylation
- This is because hydroxyl group is the recipient for glucose moiety
- Asparagine comes under N – Glycosylation due to amide nitrogen being recipient

Synthesis of creatine 00:18:32


Glycine
Arginine
Ornithine
Arginine – Glycine
Amido – Transferase
Guanidinoacetate
Aminoacids that contribute to creatine production
SAM
SAH
Guanidinoacetate methyl transferase
Creatine
ATP
ADP
Creatine kinase
Creatine Po4
Non enzymatically in muscle
Creatinine

- Note: Whenever the question asked on creatine phosphate ATP should be the answer

Synthesis of catecholamines 00:26:52



Tyrosine

H4 biopterin → H2 Biopterin

Tyrosine hydroxylase

DOPA

PLP=Pyridoxal phosphate

B6 → PLP

Dopa decarboxylase

Dopamine (1st catecholamine to be synthesized)

O_2

Cu+2

Vit C

Dopamine beta oxidase

Nor epinephrine

SAM → SAH

PNMT (Phenyl ethanolamine N-methyl transferase

Epinephrine

DOC TUTORIALS LOGIN FOR SUCCESS

BIOSYNTHESIS OF AMINO ACIDS

Nutritionally essential amino acids

Nutritionally semi essential amino acids – Arginine

Hence, body requirement of this amino acid is higher in some conditions

Nutritionally essential amino acids 00:02:17

- Mnemonic: HAM VIP LL TT
  - Histidine
  - Arginine
  - Methionine
  - Valine
  - Isoleucine
  - Phenylalanine
  - Lysine
  - Leucine
  - Tryptophan
  - Threonine

Marked ones are branched chain amino acids and these are not synthesized in body and thus essential amino acids.

Note: Tyrosine is not a part of mnemonic a nonessential amino acid.

- Essential amino acids are not usually synthesized in body
- These are readily available in diet
- Its bio synthesis in body requires 5 to 10 enzymes
- And if body is able to synthesize this class of amino acids. It is worse than good.

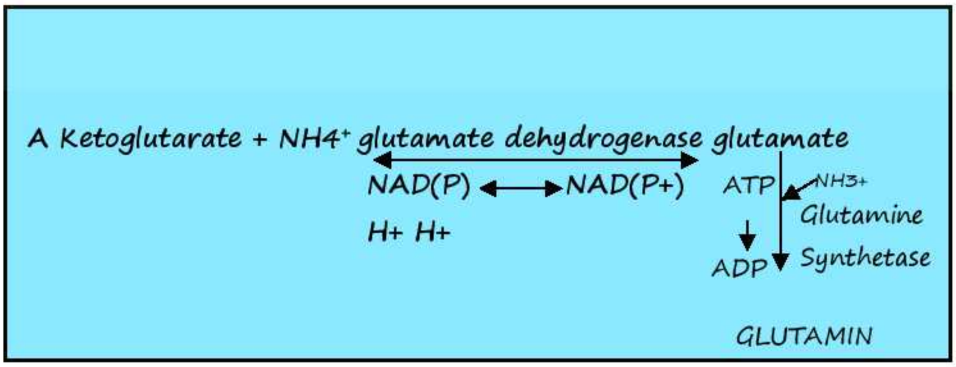
Negative survival value Q 00:05:51

- Non-essential A.A = 7AA require 1 enzyme
  1AA require 2 enzymes
  2AA require 3 enzymes
- Metabolically essential and non-essential amino acids are equally important.

Bio synthesis 00:09:38

- 7 amino acids will be synthesized using only one enzyme

1.Glutamate – single enzyme will be required


A Ketoglutarate + NH4⁺
glutamate dehydrogenase
glutamate
NAD(P)
NAD(P+)
H+ H+
ATP
NH3+
ADP
Glutamine
Synthetase
GLUTAMIN

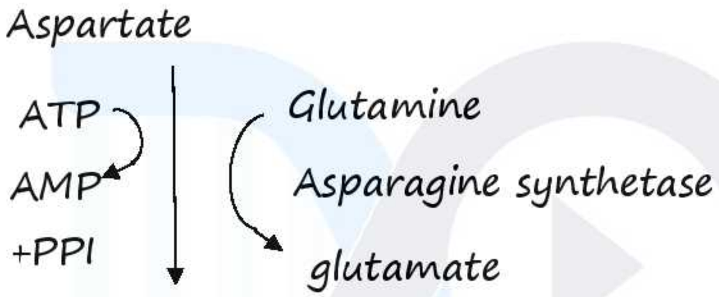
2.Glutamine – [discussed above only one enzyme required]

3.Alanine – transamination reaction will produce alanine

$$\text{Alanine} \xleftrightarrow{\text{alanine transaminase}} \text{pyruvate}$$

4.Aspartate – transamination reaction as above

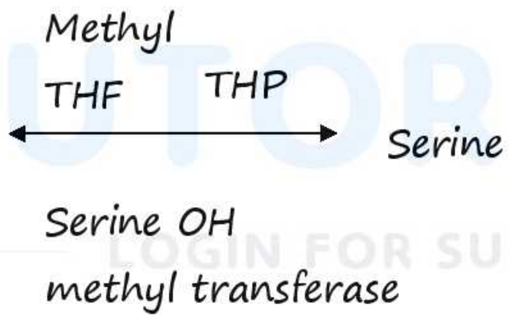
$$\text{Aspartate} \xleftrightarrow{\text{aspartate transaminase}} \text{oxaloacetate}$$


Aspartate
ATP
AMP
+PPI
Glutamine
Asparagine synthetase
glutamate

5.Asparagine

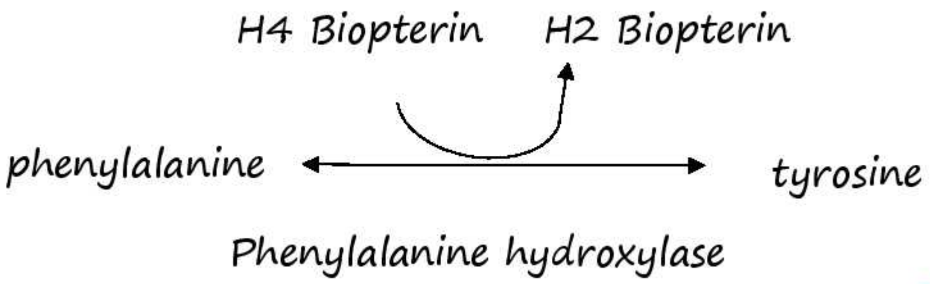
DOC TUTORIALS
LOGIN FOR SUCCESS

6.Glycine


Methyl
THF
THP
Serine
Serine OH
methyl transferase

Can also be synthesized from series of reactions from choline

7.Tyrosine readily synthesized from phenylalanine


H4 Biopterin
H2 Biopterin
phenylalanine
tyrosine
Phenylalanine hydroxylase

Synthesis using two enzymes 00:16:56

8.Cysteine

Homocysteine + serine

↓ cystathionine synthase ①

Cystathionine

↓ cystathionine lyase②

Cysteine + homoserine

[Here homocysteine is derived from methionine using SAM]

3 Enzyme usage 00:19:15

9.Serine

3 Phosphoglycerate

↓ PG Dehydrogenate

Po4 OH Pyruvate

↓ AA → Keto Acid

Transaminase

Amino acid donates amino group and converted to keto acid

Po4 serine

Po4 ↓ po4 serine hydrolase

Serine ⟷ glycine

THF → Methylene THF

10. Proline

Glutamate
ATP → ADP ↓ glutamate kinase
Glutamate po4
↓ glutamate and semi aldehyde dehydrogenase
Glutamate δsemi aldehyde
↓ spontaneous cyclisation
Pyrroline – 5 – carboxylate
NADPH +H↑ → NADP+ ↓ pyrroline 5 carb reductase
Proline

Very few genes are required for synthesis of non-essential amino acids

↓ means

Very low negative survival valve

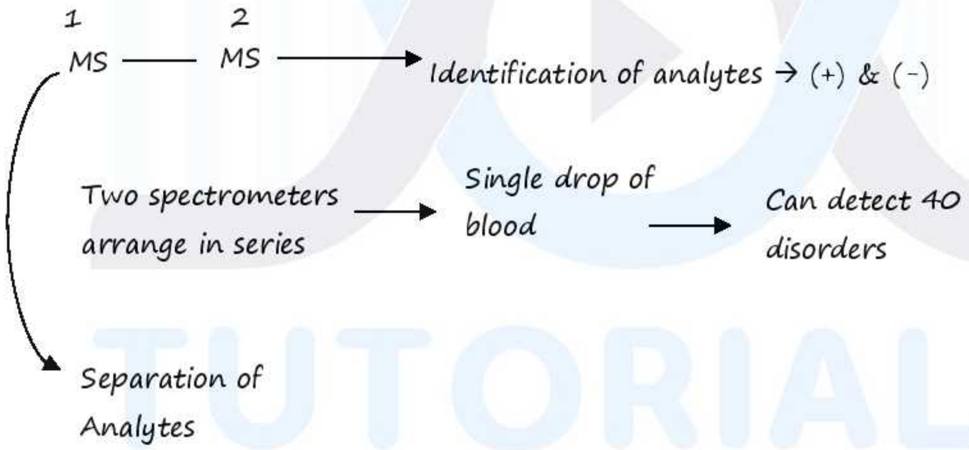
So, no need to cleave those genes during deletion.

AMINO ACIDS & CATABOLISM
– CARBON SKELETON

- Amino part of amino acid is metabolised by common pathway but carbon skeleton is different and separate pathways are involved

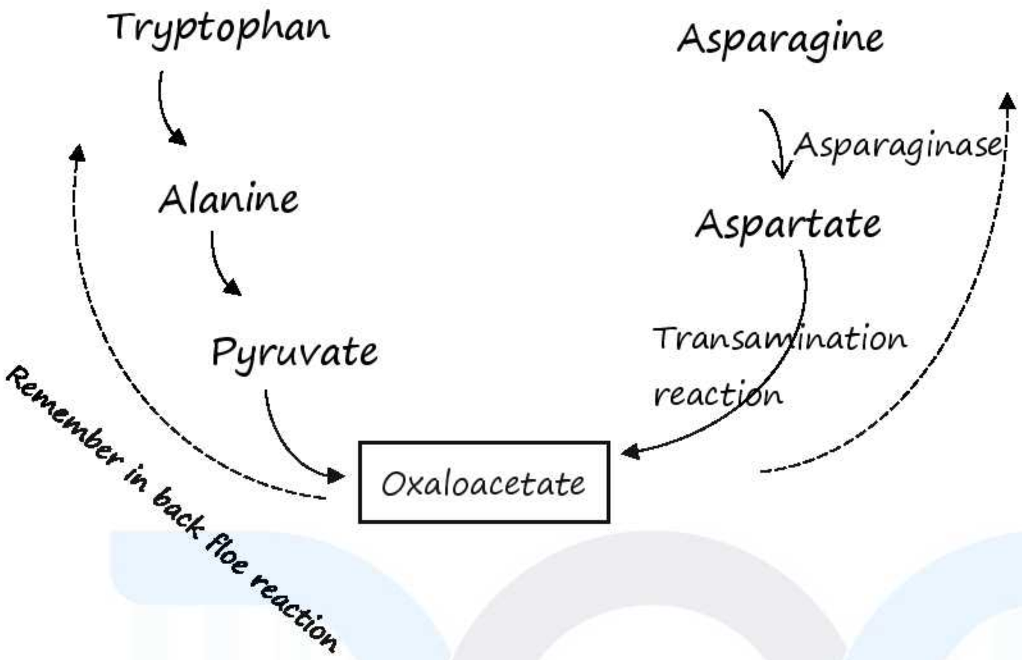
Inborn errors of metabolism 00:01:20

- We do screening for these group of disorder at birth
- By biochemical tests
- By tandem mass spectrometry (Tandem MS)


1
MS
2
MS
Identification of analytes → (+) & (-)
Two spectrometers arrange in series
Single drop of blood
Can detect 40 disorders
Separation of Analytes

- Analytes in 2nd ms can be
  - (+) of abnormal analytes
  - (–) of normal analytes
- It is confirmed by genetic analysis
- Fibroblast culture for enzyme activity
- Some of the amino acids are broken down into abnormal metabolites

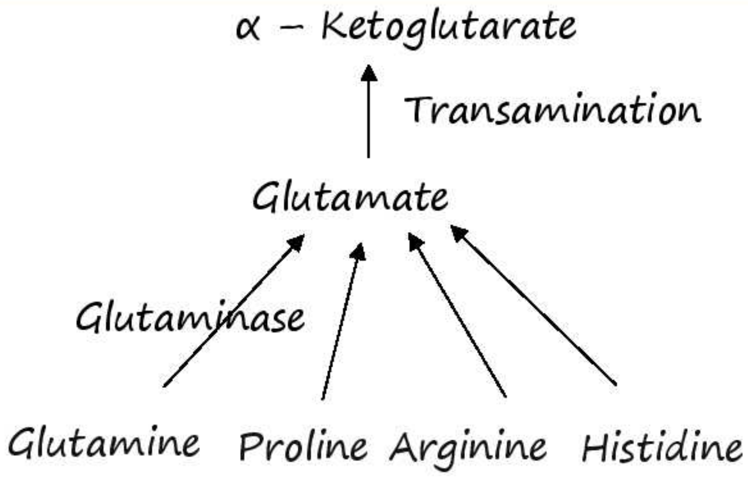
Oxaloacetate 00:05:47


Tryptophan
Alanine
Pyruvate
Asparagine
Asparaginase
Aspartate
Transamination reaction
Oxaloacetate
Remember in back floe reaction

Note:

- 18 AA are gluconeogenic (Gives rise to intermediates of krebs cycle)
- 2AA are purely ketogenic
- Anaplerotic reactions
- Intermediates of krebs cycle are produced by these anaplerotic reactions

Alpha ketoglutarate 00:08:39


α – Ketoglutarate
Transamination
Glutamate
Glutaminase
Glutamine
Proline
Arginine
Histidine

Proline

↓ Proline dehydrogenase – Deficiency leads to Hyper prolinemia (T1)

Pyrroline carboxylate

↓ Spontaneous

Glutamate γ Semi aldehyde

↓ Glutamate γ Semi eldehyde dehydrogenase deficiency lead to – Hyper prolinemia T2

Glutamate

Arginine

Urea ↓ Arginase (deficiency → Hyper arginemia)

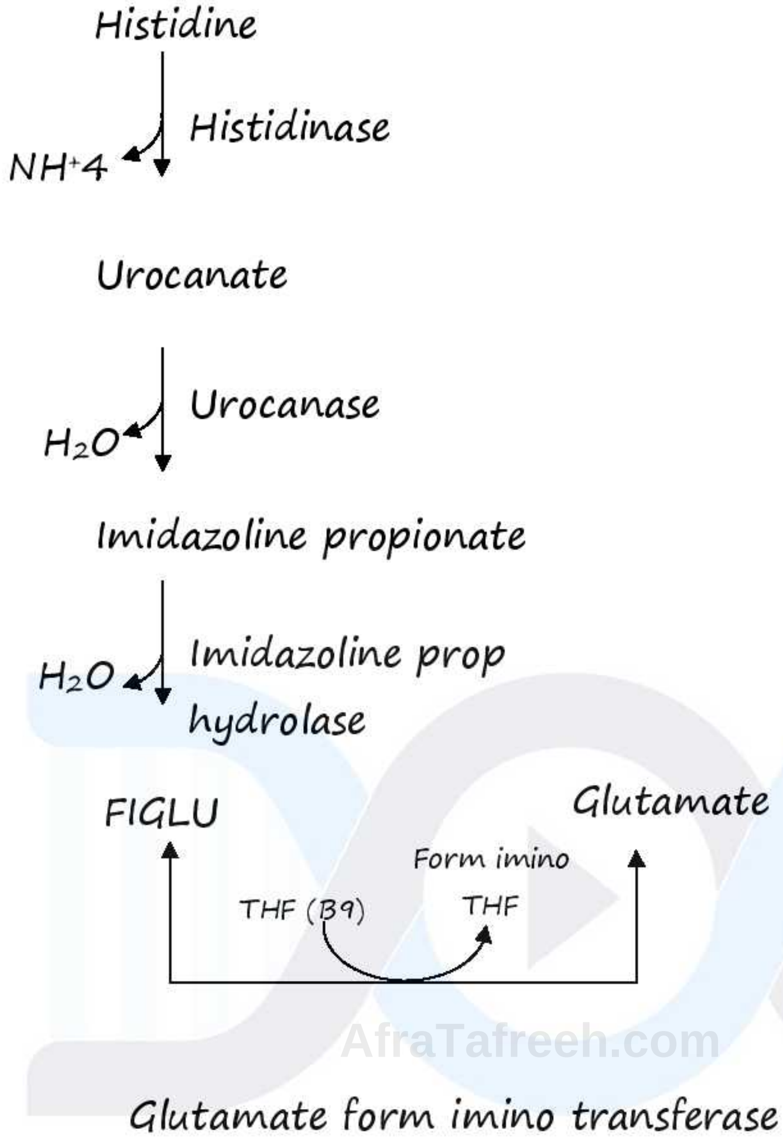
Ornithine

↓ Ornithine transaminase

Glutamate γ Semi

↓

Glutamate


Histidine
Histidinase
NH⁺4
Urocanate
Urocanase
H_2O
Imidazoline propionate
Imidazoline prop hydrolase
H_2O
FIGLU
Glutamate
Form imino
THF (B9)
THF
Glutamate form imino transferase

Deficiency of B9 Leads to → FIGIU accumulation

- Loading dose of histidine given

↓

Due to deficiency of B9

↓

FIGLU accumulates

↓

Excreted in urine

↓

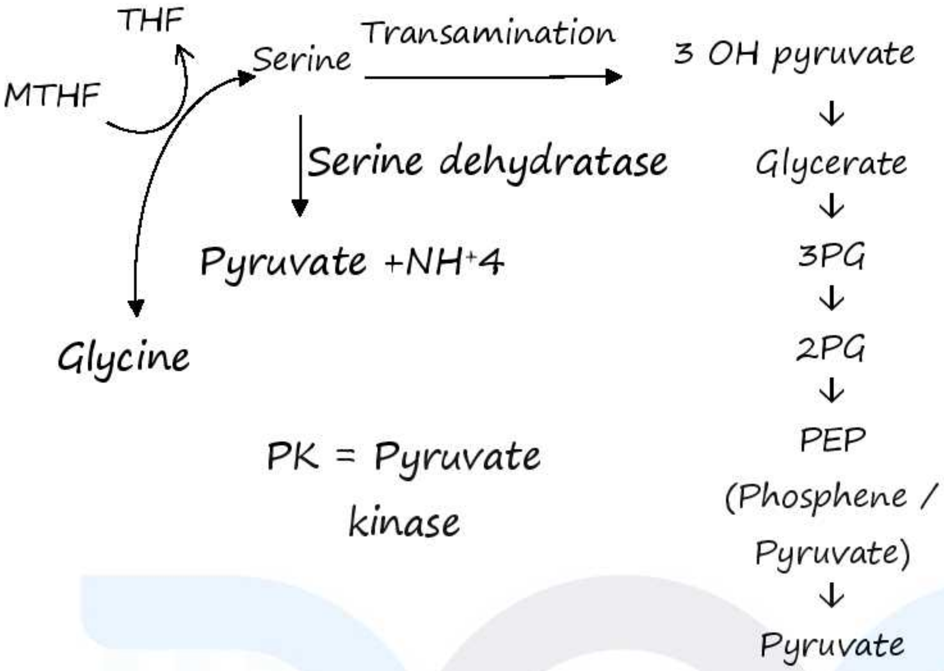
Tested as FIGLU positive in FIGLU test

↓

Indicating deficiency of B9

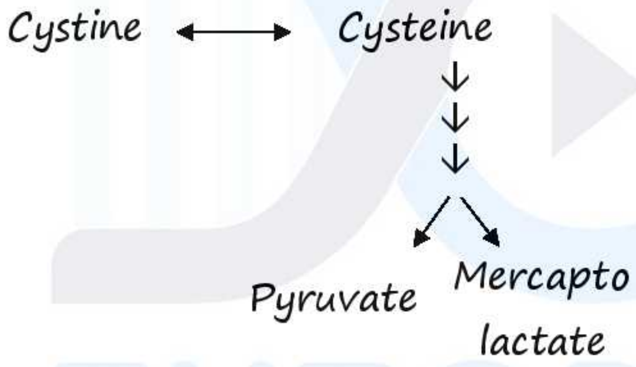
Deficiency of histidinase → Histidinemia

- Pyruvate

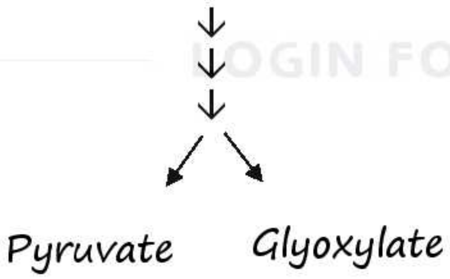

THF
MTHF
Serine
Transamination
3 OH pyruvate
Serine dehydratase
Pyruvate +NH+4
Glycine
Glycerate
3PG
2PG
PEP
(Phosphene / Pyruvate)
Pyruvate

PK = Pyruvate kinase

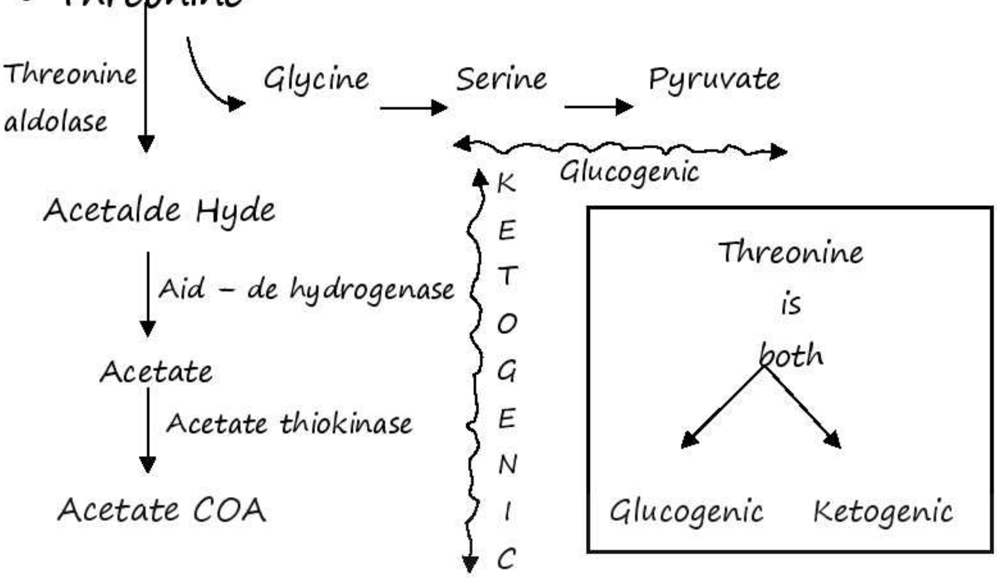
- Cystine


Cystine
Cysteine
Pyruvate
Mercapto lactate

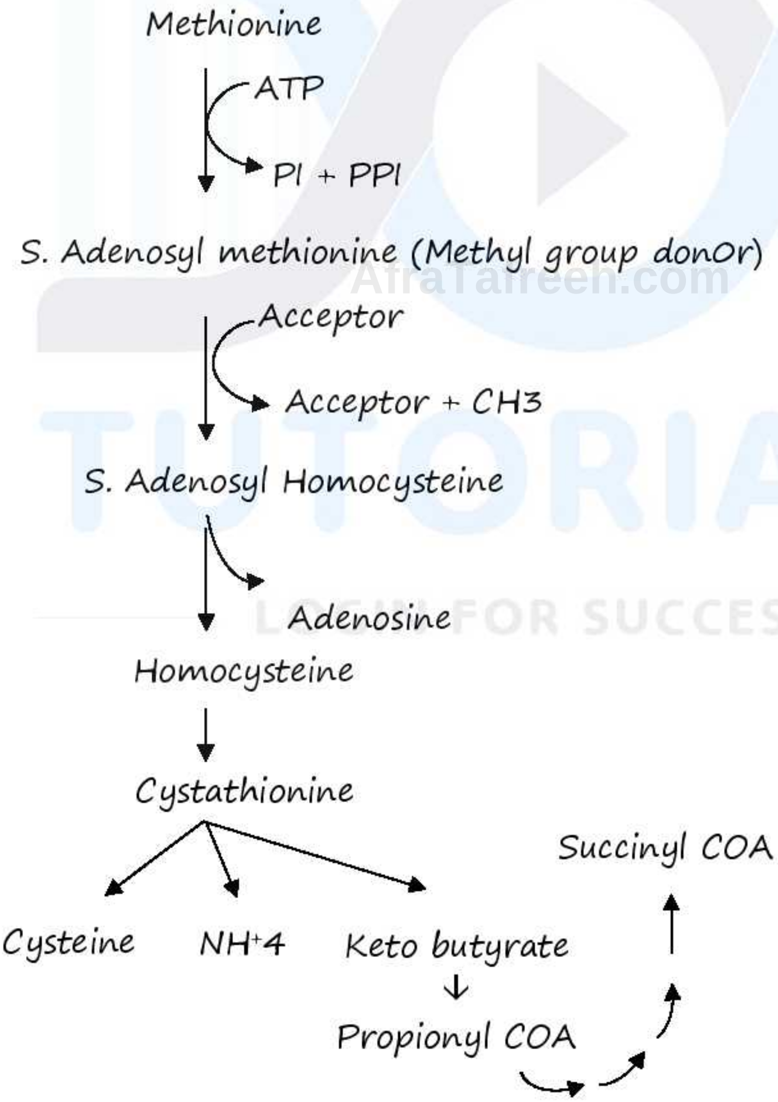
- Hydroxy Proline


Pyruvate
Glyoxylate

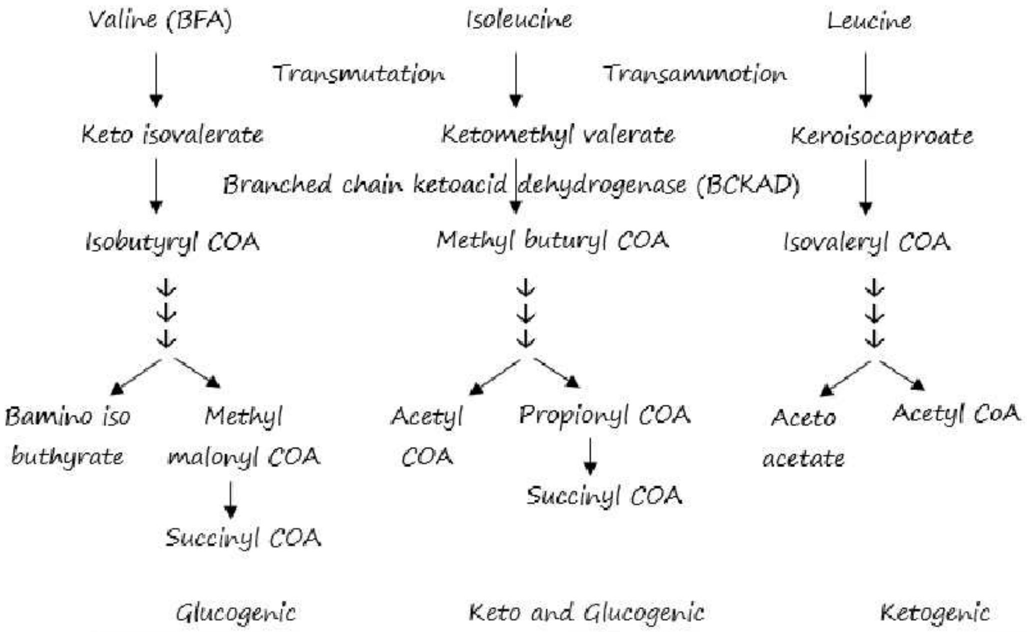
- Threonine


Threonine aldolase
Glycine
Serine
Pyruvate
Glucogenic
Acetalde Hyde
Aid – de hydrogenase
Acetate
Acetate thiokinase
Acetate COA
KETOGENIC
Threonine is both
Glucogenic
Ketogenic

- Succinyl COA


Methionine
ATP
PI + PPI
S. Adenosyl methionine (Methyl group donOr)
Acceptor
Acceptor + CH3
S. Adenosyl Homocysteine
Adenosine
Homocysteine
Cystathionine
Cysteine
NH+4
Keto butyrate
Propionyl COA
Succinyl COA

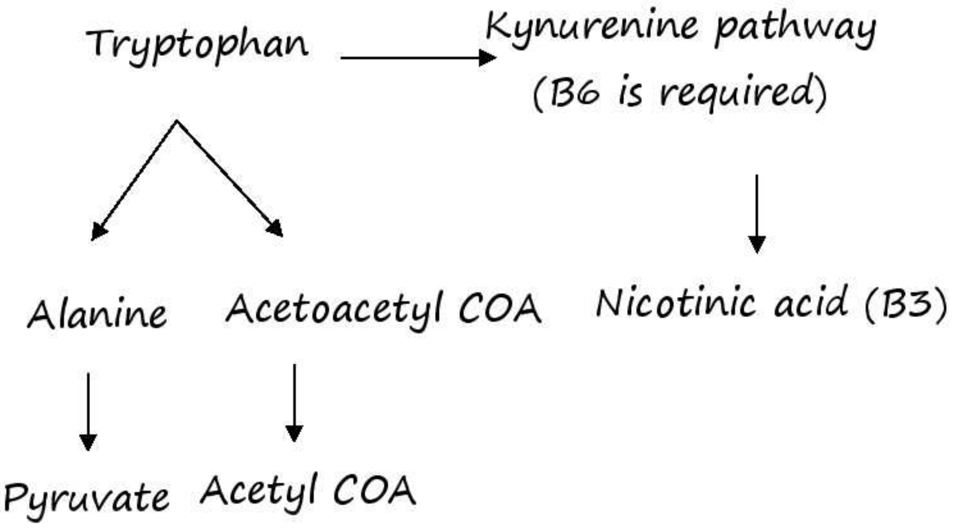
DOC TUTORIALS
LOGIN FOR SUCCESS


Valine (BFA)
Isoleucine
Leucine
Transmutation
Transammotion
Keto isovalerate
Ketomethyl valerate
Keroisocaproate
Branched chain ketoacid dehydrogenase (BCKAD)
Isobutyryl COA
Methyl buturyl COA
Isovaleryl COA
Bamino iso buthyrate
Methyl malonyl COA
Succinyl COA
Acetyl COA
Propionyl COA
Succinyl COA
Aceto acetate
Acetyl CoA
Glucogenic
Keto and Glucogenic
Ketogenic

Lysine

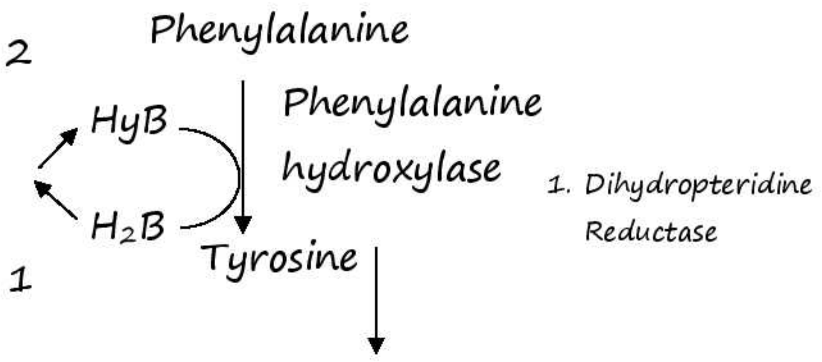
↓
↓
↓
Crotonyl COA
↓
Butanyl COA
↓
↓ Beta oxidation
↓
Acetyl COA
Ketogenic

- BCKAD deficiency leads to MAPLE syrup urine disease


Tryptophan
Kynurenine pathway
(B6 is required)
Alanine
Acetoacetyl COA
Nicotinic acid (B3)
Pyruvate
Acetyl COA

Tryptophan
Isoleucine
Threonine
} Both keto & Glucogenic

PHYENYLALANINE TYROSINE COMPLEX


Phenylalanine
Phenylalanine hydroxylase
2
HyB
H_2B
1
1. Dihydropteridine Reductase
Tyrosine

Deficiency leads to

↓

PKU (I)

(Classical)

PKU [Phenylketonuria] 00:03:11

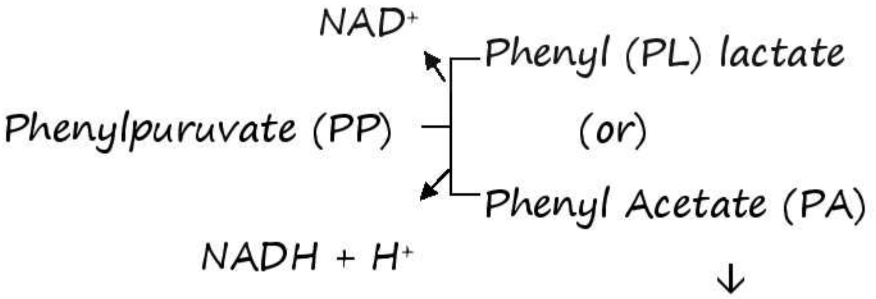
- Inherited mental retardation (preventable)

↓

by dietary restriction

of phenylalanine

Phenylalanine get converted into


NAD^+
Phenyl (PL) lactate
(or)
Phenylpuruvate (PP)
Phenyl Acetate (PA)
$NADH + H^+$

↓

in urine/sweat

gives Mousy (or)

Musty odour

DOC TUTORIALS LOGIN FOR SUCCESS

- Products synthesized from accumulated phenyl alanine are neurotoxic [PP, PL, PA]
- Guthrie test: Microbiological test – culture grows if phenyl alanine inexcess; estimates level of phenylalanine in blood
- Old term – PKU (malignant)
- New term – PKU II, III → defective recycling
  IV, V → defective biosynthesis
  - This is occurring with regard to Tetrahydro biopterin.
  - When there is recycling problem or biosynthesis problem of this coenzyme due to lack of this on e- PKU occurs.
- H_4 Biopterm is also essential for Thyrosine
  ↓
  dopa (or) cate cholamines

So even tyrosine is available from diet
↓
There is no production of catecholamines

- Tyrosine becomes nutritionally essentially AA. Which is normally a non-essential AA.

In females
↓
If no adequate treatment given or followed
↓
After her pregnancy period
↓

TUTORIALS LOGIN FOR SUCCESS

The baby will be born as severe mentally retarded which is irreversible

- It is one of the common conditions that is to be screened at newborn period in Neonatal screening

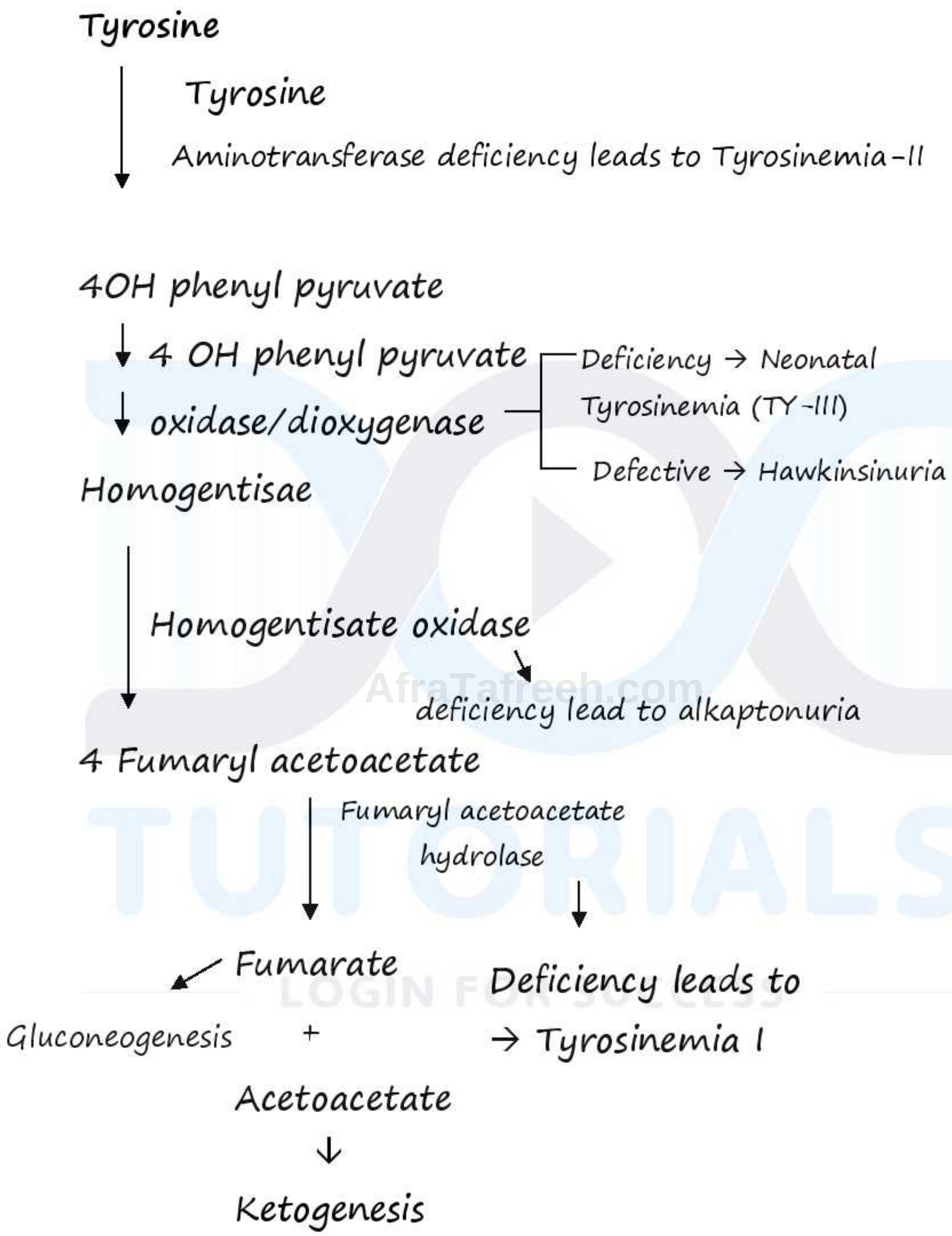
  Can use

  i. Phenylalanine levels

  ii. Enzyme activity

  iii. Tandem mass spectrometers.

PHENYLALANINE TYROSINE COMPLEX
– TYROSINEMIA & OTHERS


Tyrosine
Tyrosine
Aminotransferase deficiency leads to Tyrosinemia-II
4OH phenyl pyruvate
4 OH phenyl pyruvate
oxidase/dioxygenase
Deficiency → Neonatal
Tyrosinemia (TY-III)
Defective → Hawkinsinuria
Homogentisae
Homogentisate oxidase
deficiency lead to alkaptonuria
4 Fumaryl acetoacetate
Fumaryl acetoacetate
hydrolase
Fumarate
Gluconeogenesis
+
Acetoacetate
Ketogenesis
Deficiency leads to
→ Tyrosinemia I

- Tyrosinemia – I [Hepatorenal Tyrosinemia] or [Tyrosinosis]
  - Most severe

  - Mental Retardation + Renal stones + Boiled cabbage odour of urine
  - Progressive liver & kidney disease

↓

Presents as Rickets

(Non absorption of Ca^{+2})

NTBC/Nitisinone (treatment)

- Tyrosinemia – II
  - Keratitis + Blistering lesions of palms & soles

Occulocutaneous Tyrosinemia

or

Richner Hanhart syndrome

- Tyrosinemia – III or neonatal tyrosinemia
  - Mostly asymptomatic
  - Mild mental retardation

  NTBC/Nitisinone – [Inhibition] acts on → 4OH phenyl pyruvate oxidase or Dioxygenase

    - Usage of this drugs can convert very severe tyrosinemia I into Tyrosinemia III

- Hawkinsinuria:
  - May be asymptomatic
  - Failure to thrive d/t (metabolic acidosers)
  - Urine has Chlorine like odour or swimming pool water odour

- Alkaptonuria:
  - No developmental abnormalities, No Missed milestones, No Mental retardation — During childhood
  - Blackening of urine on standing or Blackening of Diapers — Due to exposure to oxygen A black pigment is formed

i. Erosive arthritis
ii. Back pain/stiffness
iii. Ochronosis
— in 30's & 40's

i. Homogentisate get accumulated & precipitation and forms crystals which is reason for erosions in joints.

ii. In interverterbral discs, there will be damage → fibrosis → damage → fibrosis due to erosions.

iii. Because of lower partial pressure inside the body oxidation takes longer and deposition of pigment if occurred near to the surface manifests at tight skin surfaces tip of nose, elbow, shin of tibia.

Maple syrup urine disease: Autosomal Recessive

00:21:10

- BCKAD is deficient

  ↓

  Accumulation of ketoacids

  ↓

  Backward convession to branched chain amino acids

  ↓

  BAA's convert into hydroxy acids
- So there is high levels of all these substances in plasma & urine.
- Isomer of Isoleucine → ALLOISOLEUCINE is elevated
- Leucine

  2 ketoisocaproic acid } neurotoxics in these disease
- Urinary odour → Burnt sugar/keramalized sugar
- Metabolism is normal

  But amino acids are seen in urine because of defect in amino acid transport in kidney. i.e., failure to reabsorb them.

DBMCI TUTORIALS LOGIN FOR SUCCESS

Cystinuria:

- Impaired tubular absorption of Dibasic aminoacids they are
  - Cystine
  - Ornithine
  - lysine
  - Arginine

Cystinuria

Types
↓
Autosomal recessive
↓
Defective gene → SLC 3A1

Non-Type 1
↓
Autosomal dominant
↓
SLC 7A9
↓
Associated with Incomplete Penetrance
↓
It means complete effect of the genetic defect is not seen.

Hartnup's disease

- Defect in transport of
  - Large A-A
  - Neutral A-A
  - Aromatic A-A

  } 13 Amino acids are lost in urine
- Manifest as Tryptophan deficiency

↓

Niacin deficiency (3D's)

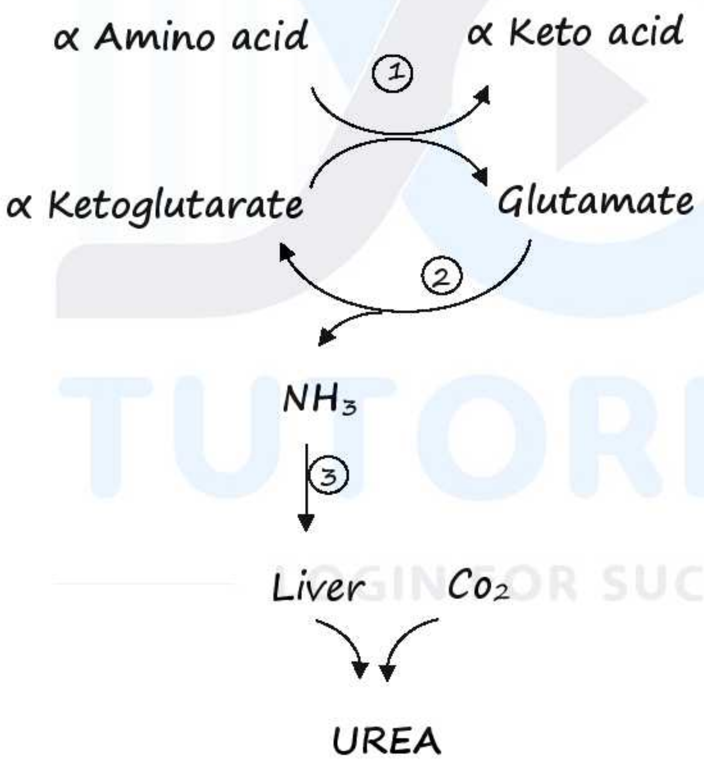
SLC6A19 gene defects

- Diarrhea
- Dementia
- Dermatitis

AMINO ACID CATABOLISM

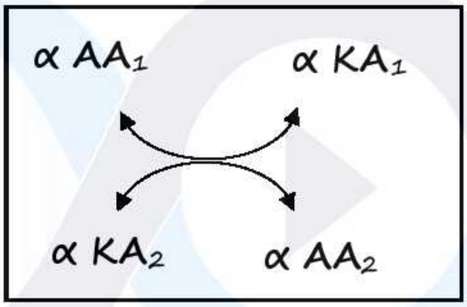
- Breakdown of amino part of amino acid is common for all aminoacids
- Discussed under - 4 stages

  1. Transamination
  2. Oxidative deamination of glutamate
  3. Ammonia transport
  4. Reactions of urea cycle


α Amino acid
α Keto acid
①
α Ketoglutarate
Glutamate
②
NH_3
③
Liver
Co_2
UREA

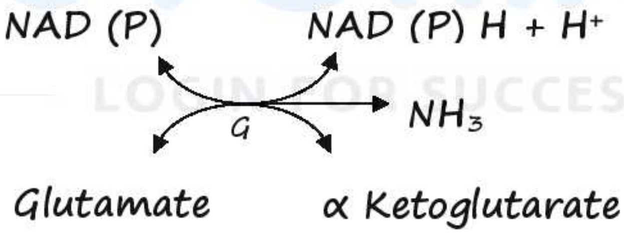
Transamination 00:04:20

- Transfer of α-amino nitrogen to α-keto glutarate (α-ketoacids)
- Forming glutamate
- Vitamin B6 is required (Pyridoxal phosphate)
- Freely reversible
- Aminoacids that don't undergo transamination are lysine, threonine, proline & OH proline


α AA_1
α KA_1
α KA_2
α AA_2

Oxidative deamination of glutamate 00:08:52

Conversion of glutamate into α ketoglutarate


NAD (P)
NAD (P) H + H^+
NH_3
G
Glutamate
α Ketoglutarate

G → Glutamate dehydrogenase

* Freely reversible

Where ammonia (NH_3) Rises increase

↓

Favourable for backward usage

i.e., combines with α-ketoglutarate

↓

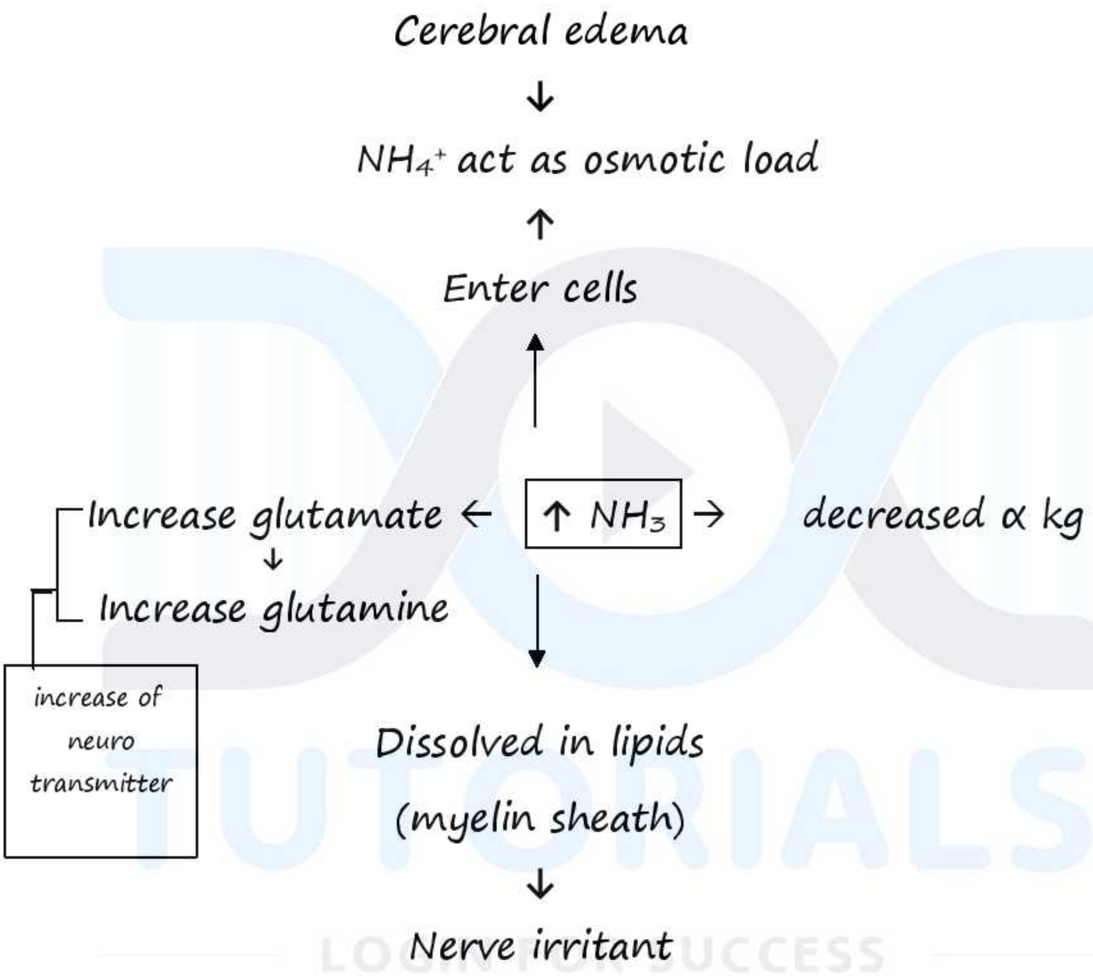
Reduced α kg

↓

Decrease in TCA cycle

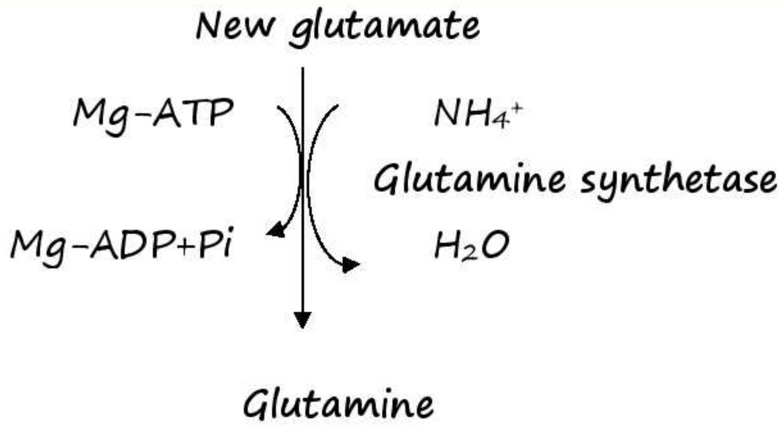
↓

Decrease ATP → Neurological signs & symptoms


Cerebral edema
↓
NH_4^+ act as osmotic load
↑
Enter cells
↑
Increase glutamate ←
↓
Increase glutamine
increase of neuro transmitter
↑ NH_3
→ decreased α kg
↓
Dissolved in lipids (myelin sheath)
↓
Nerve irritant

Effects of ammonia on CNS

Transport of NH_3 00:16:15


New glutamate
Mg-ATP
Mg-ADP+Pi
NH_4^+
Glutamine synthetase
H_2O
Glutamine

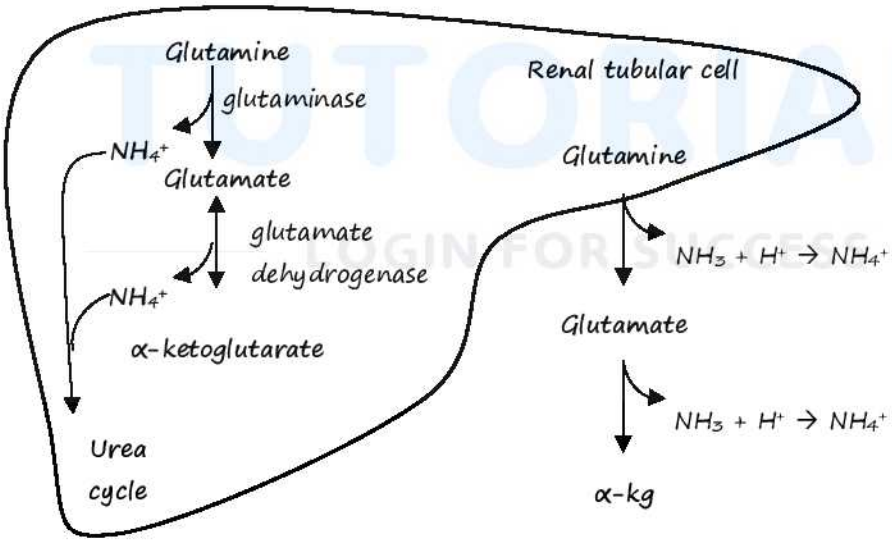
(Transport form of ammonia)

↓

Liver

- But transport form of NH_3 for skeletal muscle is Alanine Q

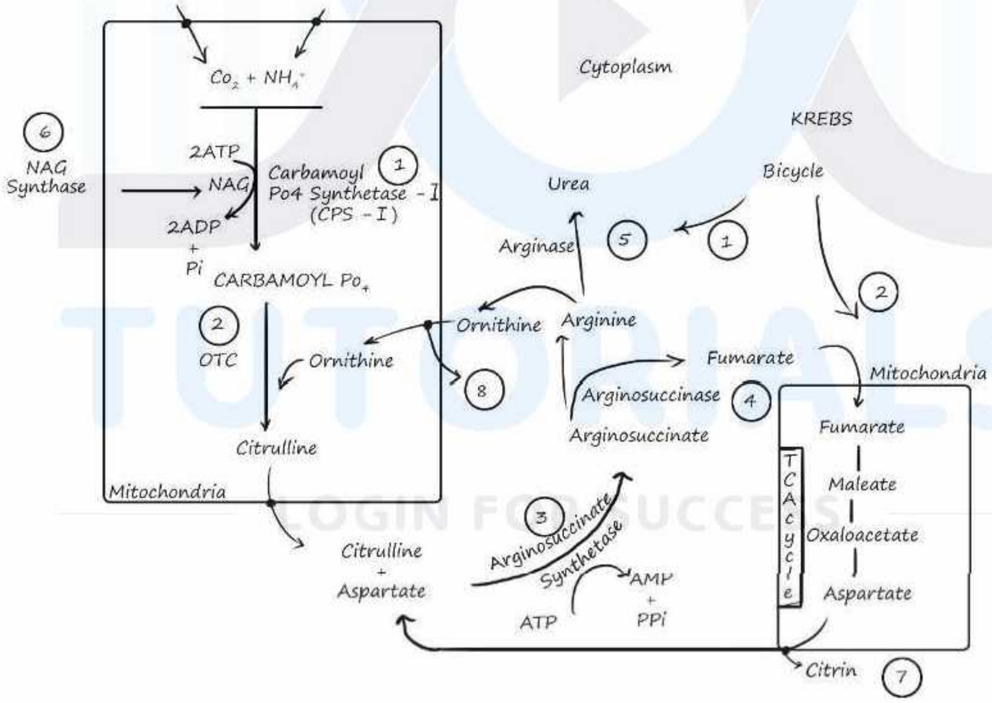
In liver 00:21:57


Glutamine
glutaminase
NH_4^+
Glutamate
glutamate dehydrogenase
NH_4^+
α-ketoglutarate
Urea cycle
Renal tubular cell
Glutamine
$NH_3 + H^+ \rightarrow NH_4^+$
Glutamate
$NH_3 + H^+ \rightarrow NH_4^+$
α-kg

* Glutamine participates in
Acid base homeostasis in
Renal tubular cells

Urea cycle: Krebs Hanseleit cycle 00:22:34

- Only in liver (also in CNS)
- Mitochondria & cytoplasm are involved
- NH_3, Co_2 & aspartate (2nd nitrogen) → Donor
- Urea & ornithine both are generated
- CPS-I rate limiting enzyme
  - Targeted for regulation
- 4 ATP equivalents are required for a cycle
- N acetyl glutamate (NAG) required as a coenzyme for CPS-I


Co₂ + NH₄⁺
⑥
NAG Synthase
2ATP
NAG
2ADP + Pi
Carbamoyl Po4 Synthetase - I (CPS - I)
①
CARBAMOYL Po₄
②
OTC
Ornithine
Citrulline
Mitochondria
Cytoplasm
KREBS
Bicycle
Urea
Arginase
⑤
①
②
Ornithine
Arginine
⑧
Fumarate
Arginosuccinase
④
Arginosuccinate
Mitochondria
Fumarate
Maleate
Oxaloacetate
Aspartate
TCA cycle
③
Arginosuccinate Synthetase
Citrulline + Aspartate
ATP
AMP + PPi
Citrin
⑦

- Arginosuccinase is called as arginosuccinate lyase
- OTC – Ornithine Trans Carbamoylase
- CPS-I & CPS-II are isoenzymes
  - CPS-I → Mitochondria, urea cycle
  - CPS-II → Cytoplasm, pyrimidine synthesis

- Citrin = aspartate glutamate transporter

- Bridge between urea cycle & TCA cycle
  - ^{Q}Substrate → Fumarate
  - ^{Q}Aminoacid → Aspartate

DISORDERS OF UREA CYCLE

Disorders (refer numbers mentioned above)

Hyper ammonemia → severe①,②

Mild-moderate

1) CPS1: - Deficiency leads to hyper ammonemia type 1
2) OTC: - Deficiency leads to hyper ammonemia type 2

Reaction 1 does not happen

↓

Ammonia will accumulate because of no conjugation

Reaction 2 does not happen

↓

Carbamyl Po4 accumulates

↓

Exerts negative feedback on reaction 1

↓

So reaction 1 does not happen

↓

NH3 accumulates

- However, if reaction two is completed

↓

Feedback is lesser across membranes

↓

So, severity of hyperammonemia is less

3) Arginosuccinate synthetase: deficiency lead to → citrullinemia - I
4) Arginosuccinate lyase: Deficiency lead to → Arginosuccinic aciduria
5) Arginase deficiency lead to → hyper argininemia
6) NAG Synthase deficiency lead to → condition similar to hyperammonemia - I
7) Citrin malfunction or not working lead to → citrullinemia – II

Because aspartate cannot be transported and thus citrulline will be alone leading to accumulation

8) Defect of ornithine transporter lead to hyperammonemia
Hyperornithinemia
Homocitrullinemia

Therefore, carbamyl Po4 converts into homocitrulline

TUTORIALS LOGIN FOR SUCCESS

Triple H syndrome 00:08:01

All are

← Autosomal – Recessive →

Expect OTC deficiency X linked recessive.

Orotic Aciduria 00:09:55

Carbamoyl phosphate accumulation

↓

Leaks into cytoplasm

↓

Gets converted into Orotic acid

↓

Cycle stops (Blocked)

Carbamoyl P entering into pyrimidine pathway

↓

Orotic acid accumulates

- In case if CPs – I or NAGs deficiency there is no chance of orotic aciduria because carbamoyl Po4 production is not possible.

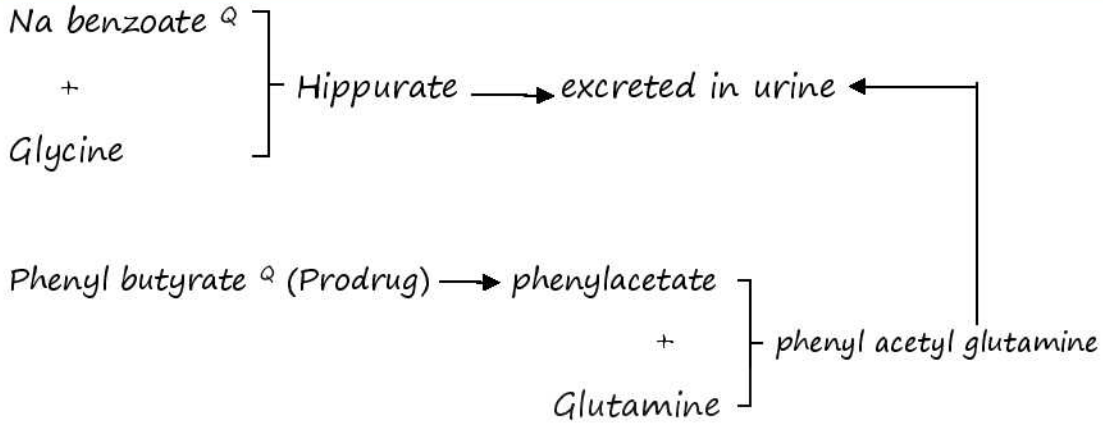
Management 00:11:31

To decrease NH4/Aminoacid in body

- Decrease the protein intake
- Increase gut motility (less time for absorption) – give lactulose
- Sterilize the gut (gut bacteria is source of NH4) – give neomycin

To excrete nitrogen /NH3 by alternate routes

00:13:02


Na benzoate Q
+
Glycine
Hippurate
excreted in urine
Phenyl butyrate Q (Prodrug)
phenylacetate
+
Glutamine
phenyl acetyl glutamine

Bypassing the block 00:15:41

Arginine supplementation: - bypass block at ③ and ④

Arginine synthesis cannot occur

when ③ or ④ is blocked

↓

Arginine supplements given

↓

Splits into ornithine

↓

Proceeding occurs at reaction ②

↓

Citrulline enters cytoplasm

↓

Feedback is less across membranes

↓

Severity of hyper ammonemia is very less/mild

N carbamoyl glutamate (similar to NAG)

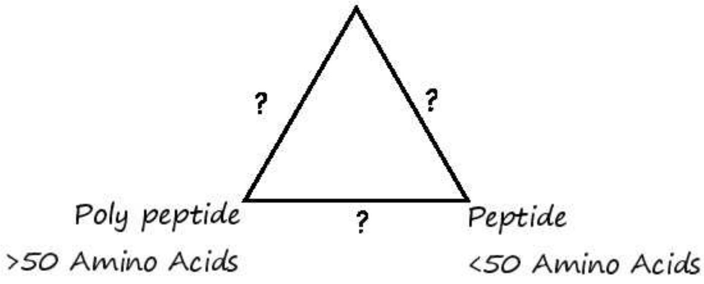
↓

Can act as coenzyme for CPS – I.

PROTEINS STRUCTURES

Protein structure

Protein is a polymer of aminoacids



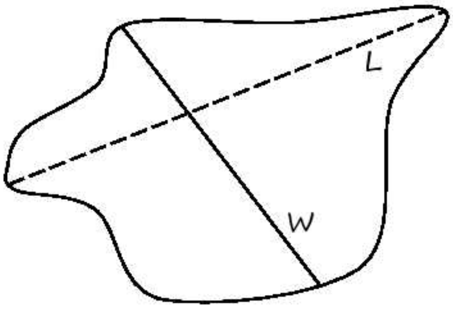
If polypeptide has $\boxed{\text{>100 Amino Acids}}$ → Protein

Integral (vs) Soluble proteins 00:04:20

Integral proteins	Soluble proteins
↓	↓
Present in membranes	Present superficially
↓	↓
In-order to take out protein we have to destroy the whole membrane	Taken out easily from membrane

Structures based on axial ratio 00:03:58

- On basis of axial ratio = $\frac{\text{Longest diameter (L)}}{\text{Width (W)}}$



- Fibrillar proteins $\frac{L}{W} > 10$
- Globular proteins $\frac{L}{W} < 3$

Function of protein is decided by structure (or) form 4 orders are described

Order	Description	Nature
1° structure intact after denaturation	Sequence of Amino Acids	Covalent peptide bonds
2° structure easily destroyed by denaturation	Geometrical shapes formed by contiguous Amino Acids α helix, β sheets, loops, turns, bends. typically, of 3 to 30 Amino Acids	Non covalent bonds[Q] - Hydrogen bonds - Ionic forces - Vander waal forces - Hydrophobic interaction

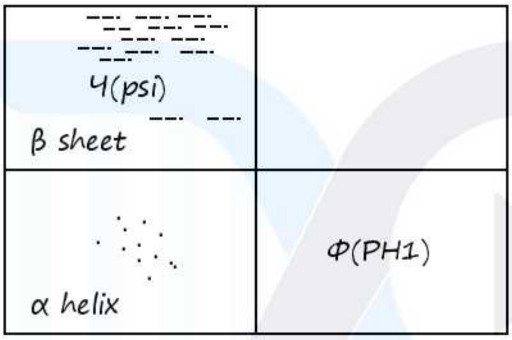
<table>
<tr><td>3° structure</td><td>Spatial arrangement of all 2° structures arranged around each other</td><td>Non covalent > covalent (Disulphide bonds) (C.B)
- Hydrogen bonds
- Ionic forces
- Vander waal forces
- Hydrophobic interaction</td></tr>
<tr><td colspan="3">3° structure describes only one polypeptide
In case of some proteins there are more than one polypeptide
↓ Called as
Oligomeric proteins</td></tr>
<tr><td>4° structure</td><td>3D/spatial arrangement of various polypeptides around each other</td><td>Covalent< non covalent
- Disulphide
- H.B, I.F, V.W.F, HFI
- Condensation bonds</td></tr>
</table>

Since, condensation bonds are cross links formed between side chains

- As non-covalent bonds are major in 2°, 3°, 4° structures these can undergo denaturation easily

Rama Chandran Plot 00:16:31

- It describes permissible combinations on a graph predicts 2° structure of protein.
  - **Phi Angle:** angle between α-carbon & nitrogen
  - **PSI angle:** Angle between α-carbon & carbonyl carbon



- Thus, this plot helps in predicting 2° structures

Super Secondary Structure 00:21:55

- Distant aminoacids come together within a functional domain.

Some 2° structures come together.

↓

Form a biologically functional domain, is called super 2° structure

Example- Rossamann fold

- Commonly seen in enzymes having the function
- Of DEHYDROGENASE

- **Ex:** lactate dehydrogenase
- Malate dehydrogenase
- Succinate dehydrogenase
- Present b/w 2° & 3° Structures.

POST TRANSLATIONAL MODIFICATION

- Folding
- Covalent changes

Folding 00:01:37

- Cytoplasm/Endoplasmic reticulum/Gogi etc.
- Protein contains information for folding but They are unable to fold on their own because size related problem and Multiple possibility with provided information
- If in addition need another group of proteins that tells group of proteins that tells which is correct folding (Auxiliary protein)

Auxiliary proteins

- Chaperons – Meade up of 2 types
  - Chaperonins
  - Heat shock proteins (Rescue proteins)
  - Helps out misfolded proteins
  - Heat shock proteins unfold the misfolded proteins
- Protein disulphide isomerase
  - Migrates disulphide bonds to achieve the stable conformations
- Peptidyl prolyl cis trans isomerase
  - Shifting of small structure in amide part to cis or trans orientation
- Calnexin
- Calreticulin

- GRP – 94 (Glucose regulated protein)
- BIP (Ig heavy chain binding protein)

Covalent changes 00:07:32

- e.g. Collagen → Type 1

  ↓

  (3 polypeptides)

Cytoplasm 00:08:13

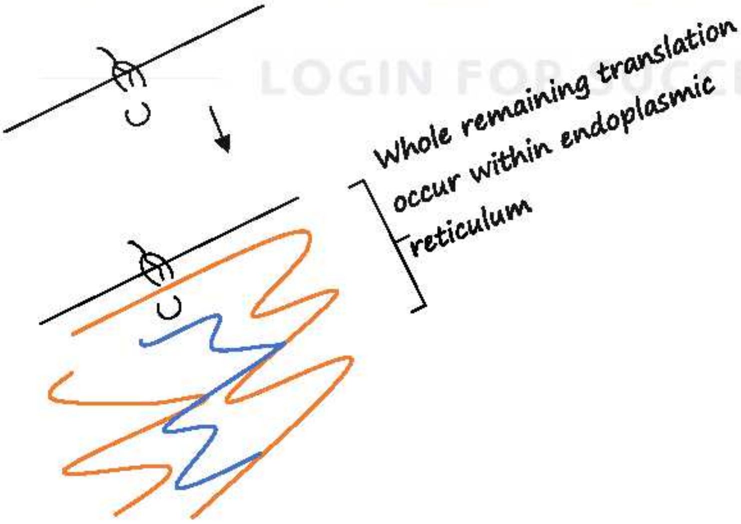
- Translation for messenger RNA occurs
- During translation a unique sequence get translated

↓

Signal peptide/leader sequence

↓

Blocks the translation migrates the translational machinery to ER

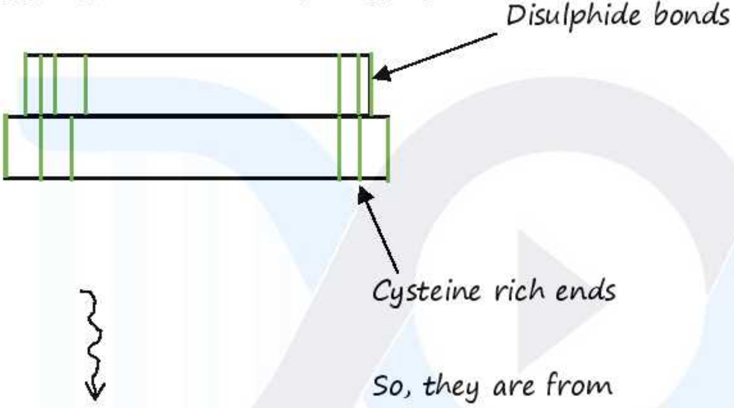

Whole remaining translation occur within endoplasmic reticulum

Endoplasmic reticulum 00:10:12

- Translation completes

  ↓

- Cleavage of leader sequence occurs
- Hydroxylation of lysine & proline by lysyl/Prolyl hydroxylase
- Glycosylation
- Aggregation of 3 polypeptides



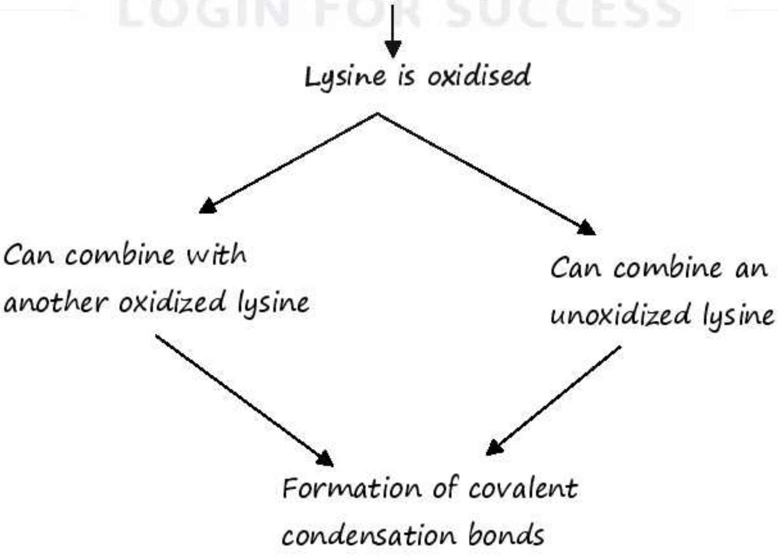
Secreted into E. C. M

So, they are from disulphide bonds

AfraTanveer.com
DOC TUTORIALS
LOGIN FOR SUCCESS

Extracellular matrix 00:13:57

- Cleavage of cysteine rich ends
- Oxidation of lysine by lysyl oxidase (Copper is co factor)



Lysine is oxidised

Can combine with another oxidized lysine

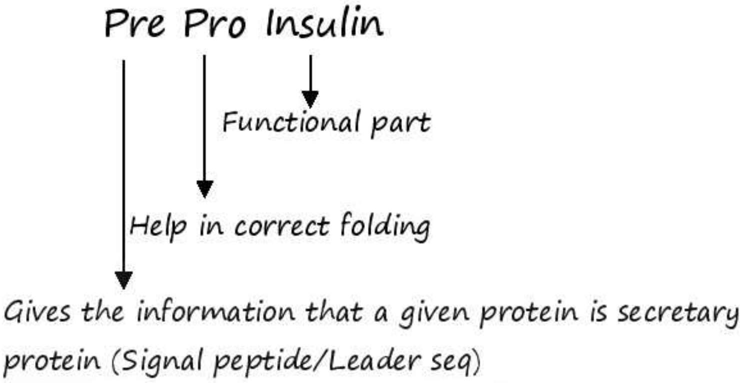
Can combine an unoxidized lysine

Formation of covalent condensation bonds

- Final folding of 3 polypeptide chains

Insulin

- At end of translation



Pre Pro Insulin

Functional part

Help in correct folding

Gives the information that a given protein is secretary protein (Signal peptide/Leader seq)

Conformational disorder 00:20:32

- Problem in post translational modification

Example

- Infections → prion diseases
- Dietary deficiency → Scurvy (Lack of co enzyme)
- Degenerative disorder → Alzheimer's
- Inherited disorder → Severity of Beta thalassemia

α Chain folding protein (-)

TUTORIALS LOGIN FOR SUCCESS

COLLAGEN

Collagen 00:00:45

- A very important preterm in extracellular matrix
- 1/3rd of body protein is collagen [25%]
- 28 different types are there
- 30 different genes.

Collagen – I 00:02:07

- Present in extracellular matrix of non-cartilaginous tissue.
- Provide strength to tissue

  It forms triple helix structure

  - Each polypetide is wound in it handed direction
  - If winding around each other, they are wound in right-handed direction.

- Because of opposite unwinding of polypeptide and complex structure unwinding of collagen is very difficult

  Common structure of polypeptide [Gly – X – Y]

  n X 1000 (Repetition)

  100-150 copies of proline

  100-150 copies of OH-proline

- Most common amino acid in collagen – Glycine

  2nd most common – Proline

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- Because collagen forms tight helix

  ↓

  It has sharp turns

  - So, it is not easy for other amino acids to settle
  - Glycine is so small & it can fit
  - Proline is responsible for sharp turns making it tight.

- 3 Polypeptide are wound around each other & bound together by 2 types of bonds

  i. Weak, Fragile, Large in number – Hydrogen bonds

  ii. Strong, less in number covalent cross linking

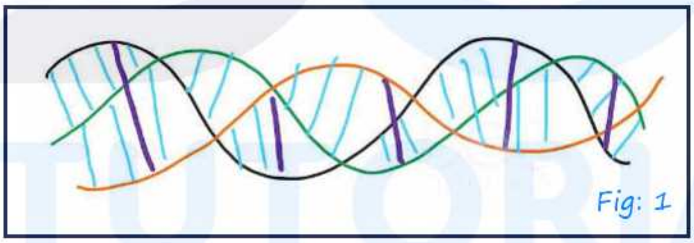

Fig: 1

- OH proline
  OH lysine] are responsible for numerous hydrogen bond formation

- Thickness of this triple helical structure – 1.4nm
- Collagen is laid down in
  - All the subsequent layers which are laid down are shifted by quarter position
  - 2nd layer is quarter position ahead of 1st layer

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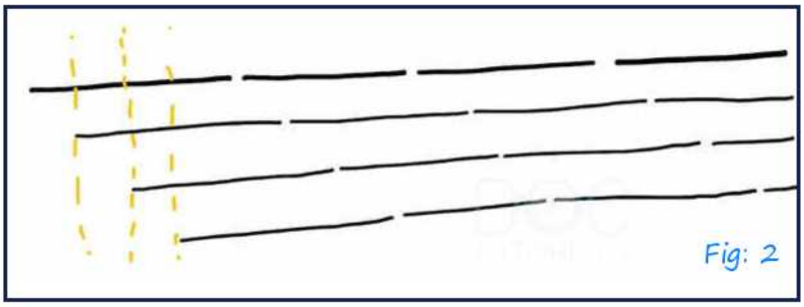

- This is called Quarter Staggered Arrangement because of this striated appearance on microscope
- Extra strength provided to the ECM because different layers will support the gap in one of the layers.
- It provides clear path for a light to pass through

Structure formed by collagen

- Fibril = seen in I, II, III, IV
- Network seen in IV, VIII
- Transmembrane seen in XIII, XVII
- Anchoring fibrile seen in VII
- Beaded filaments in VI, XXVI
- FACIT (Fibril Associated Collagen with Interrupted Triple helices) IX, XII, XIV, XVI

Collagen I	In non cartilaginous connective tissue including - Bone - Tendon - Skin
Collagen II	Found in cartilage & Vitreous humor
Collagen III	Found in extensible tissues – Skin, Lungs, Vascular system
Collagen IV	Forms network like structure Found in basement membrane kidney, Inner ear, Eye
Collagen VII	Function of anchoring fibrils At junction [Dermal – Epidermal junction]

Inherited Disorders of Collagen 00:22:16

- Osteogenesis imperfecta – Blue sclera + fragile bones (Pseudo battered baby syndrome)

  ↓

  Bones are at different phases of healing

  - 4 different subtypes: (both collagen I & II may be defection)

- Ehler Danlo's syndrome: Cutis hyperlastica
  - Rubber man syndrome
  - Hyper extensibility of skin
  - Hyper flexibility of joints

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- 6 different types
- Some of them have fragile blood vessels.

- Alport's syndrome: Colagen 4A5 gene defective

  [Collagen type 4 fibril no. 5]

  - Present as nephritic syndrome (glomerulus)
  - SNHL (inner ear)
  - Visual defects (eye)

- Menke's disease: ATP 7A gene defective

  Lysysl oxidase is the enzyme affected

(∵ Cu^{+2} is required for lysye oxidase which is transported by ATP TA)

Kinky hair (Characteristic)

Poor development

Skin pigmentation

- Epidermolyst bullosa: Dystrophic type

  Col VII defective → easy brusising, bulla/vesicle formative

  [∵ Simplex involves keratin 5 deficiency]

- Scurvy "Not an inherited disorder"
  - Dietary deficiency of vitamin – C

- Marfan's syndrome:

  Abnormal deposition of Normal collagen

  Fibrillin I is defective

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↓

It regulated TGF – β

Arachnodactyly, dislocated lens

TGF – β is abundant in

- Periosteum of long bones → extralong limbs (arachnodactyly)
- Zonules of lens → dislocations of lens
- Large blood vessel → Coarctation of aorta

∵ Deficiency of fibrillin – II leads to congenital contractural arachnodoctyly

- Good pasteur's syndrome

Abnormal immune response againsts normal collagen.

SEPERATION OF PROTEINS

- On the basis of solubility but in method main drawback is pure form of protein is not obtained.
  - It gives crude estimate of amount in mixture/solution.
  - Not used now a days.
- Precipitation by heavy metals $ZnCl_2/ZnSO_4$

  These are used for separation of proteins
- Salting out phenomenon:

  Globulins → get precipitated at ½ saturation

  Albumin → get precipitated at full saturation

  By using salts commonly NH_4Cl
  - It words on concept of Disruption of hydration shell

    ↓

    Removal of water molecules around the protein
  - Salt is present in small amounts and is known to increase the solubility

    ↓

    If concentration of salt increases

    ↓

    It absorbs water

    ↓

    Protein get precipitated

- On the basis of molecular size

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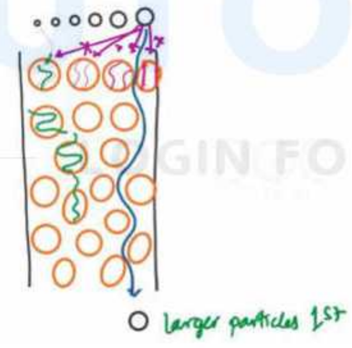
- It is of use in some special conditions.

Dialysis

- Semiporous membrane is present which separate larger & smaller particles.
- Ex:
  i. CKD/ESRD
  ii. Digitalls toxicity
  iii. Myoglobin in blood following crush injury (to prevent entry into kidney)

Gel filtration Chromatography:

- Gel filtration chromatography: also called Size-exclusion chromatography.
- Large particles are separated in beginning.



Beads are present with varying sizes of pores
↓
Larger particles cannot pass through the pores
↓
So they pass out in between beads
Whereas smaller particles enter pores of

their appropriate size thus travels longer

Smaller Particles takes time to clear out through beads	Larger particles are separated out first

#Ultra-centrifugation

- # ultra-centrifugation: High speed separation based on sedimentation coefficient

  Larger sedimentation coefficient → Separation earlier
- Not useful for proteins but can be for nucleic acids, separation of subcellular organelles can be done by this.

On the basis of molecular charge: 00:17:17

Ion exchange chlomatography:

- For charged proteins
- For positive proteins negatively charged column and vice cersa

  ↓

  Particles are held by column everything else is cleared out

  ↓

  Ph is changed

  ↓

Held particles are released

↓

Purest form of protein can be obtained

High pressure liquid chromatography

- Time taken is shortened (lesser)
- Can use size-based method, also has charged column model.

Electrophoresis:

- Types – agarose get electrophoresis
  Polyacrylamide gel electrophoresis
  Isoelectric focusing
- The during for the above methods charge but separation criteria is different

Agarose

Gel – E

↓

Based on charge to mass ratio q/M

↓

Theoretically, can be used for proteins but it is used for nucleic acids

↓

Commonly useful for larger particles

Polyacrylamide Gel (P.A.G.E)

Native Page
↓
By q/m
↓
When we want to separate a biologically functional protein. It will be separated intact

SDS page
↓
By basis of mass
↓
Protein is denatured
↓
Used for estimation of the Relative mass

Isoelectric 00:24:39

Focusing (For proteins, even Amino acids)
↓
By basis of
ISO electric PH

Feasible for the smaller number of proteins but for large number of proteins clubbing process is done.

Isoelectric focusing is done first
↓ Followed by
SDS - PAGE

Two dimensional electrophoresis

SDS page

- Sodium dodecyl SO_4 (detergent)
- Protein is denatured
- During denaturing disulphide bonds → (Broken by using β mercaptoethanol) are broken

- Imparts a very large negative charge to protein proportional to mass.
- Proteins achieve rod like shape

On basis of Relative mass

To attain highly purest form:

- On the basis of affinity binding:
  - i. Affinity chromatography
    - Beads have property of affinity towards targeted molecule
    - Thus trapped
  - ii. Precipitation by antibodies
    - Similar to ELISA where there is only quantification
    - Here, after the process antibodies and proteins are separated to obtain it.

NUCLEOTIDE PROPERTIES

Physiological Functions of Nucleotides 00:00:31

- Building blocks of DNA/RNA
- Energy transducers – ATP/GTP
- Second messenger – CAMP, CGMP
- Co-enzymes – NAD^+, $NADP^+$, FMN, FAD
- One carbon metabolism – SAM (S-Adenosyl – Methionine) (Other important molecule for one carbon metabolism – Tetra hydofolate (THF)
- Transporter
  - Hexoses – ex: UDP – Glucose, UDP-galactose
  - Uronic acid – ex: UDP – Glucuronic acid
  - Sulphate – ex: Adenosine – 3 – phosphate – 5 – sulphate
- Dietary purines and pyrimidines (Don't reach blood)

↓

Taken by enterocytes and degraded

↓

Degradation products reach blood

Purines
↓
Uric acid

pyridines
↓
Ammonia (NH_3)
Carbon dioxide (CO_2)
Beta – amino acids

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Nucleotide analogues/Modified nucleotides

00:06:57

- Can enter blood so have many clinical applications
- Mechanism of action, MOA:
- Chain disruption of DNA/RNA
- Because of similarity they can block the enzymes

Nucleotide analogue	Use
Allopurinol	Gout
5-flurouracil	Chemotherapy
5-Azacytidine	Immunosuppression
Nucleoside/Nucleotide reverse transcriptase inhibitors (NRTI's)	Anti-retroviral therapy (ART) for HIV infection

Interaction of Nucleotides with light 00:09:53

- Absorption maxima – Wave length at which the maximum absorption of light occur for a particular substance
- Absorption maxima for nucleotides – 260nm (UV range) → Not visible to naked eye

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Uses of measuring absorption at 260nm 00:11:47

- Quantification
- Quality of solution/assessment of purity
- Hyperchromic effect

Quality of selection 00:12:05

- It tells about whether the solution contains DNA only or DNA with protein
- Compare via the ratios of 260/280 absorption

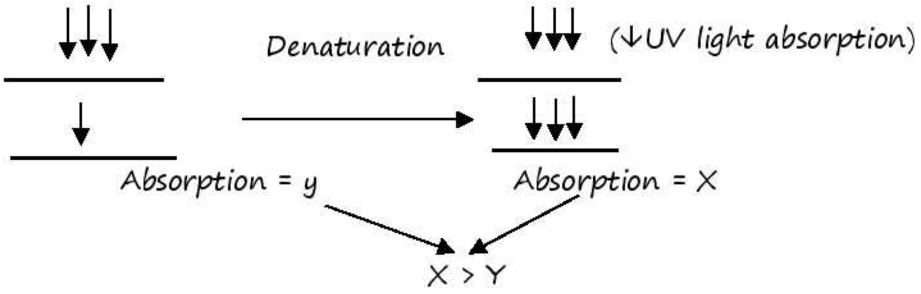
Quantification 00:12:34

Type of nucleic acid	Conversion factor for OD = 1
Ds DNA	50ng/µl
Ss RNA	40ng/µl
Ss DNA	33ng/µl
Oligonucleotides	20-25ng/µl

- Ex: If the OD of a solution with ds DNA when absorption measured at 260nm is 0.8
- 0.8 x 50ng/µl
- 40ng/µl (Concentration of ds DNA in the given solution)

Hyper chromic effect 00:14:31

- On denaturation the absorption of ds DNA increases known as hyperchromic effect


Denaturation
(↓UV light absorption)
Absorption = y
Absorption = X
X > Y

- The UV light absorption of ds DNA when compared to denatured DNA is less because the upper strand decreases the exposure of lower strand

Uses of hyperchromic effect 00:16:16

- Determines denaturation temperature of DNA

↓

Also known as melting temperatures (TM) because on denaturation viscosity of DNA ↓

- Identifies denaturing agents

Note

- Infra-red light →>700nm wave length → Low energy
- Ultra violet light →<400nm wave length → high energy

↓

Disrupts the bonds in nucleotides

↓

Mutation

↓

∴UV light is mutagenic

- The closer the wave length to 260nm more is the mutagenic potential

DNA - 1

Polynucleotides 00:00:16

i. DNA

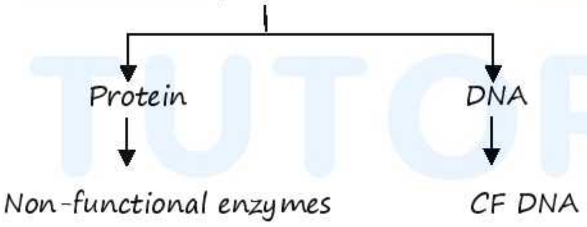
ii. RNA

DNA (2' deoxy ribose nucleic acid) 00:00:51

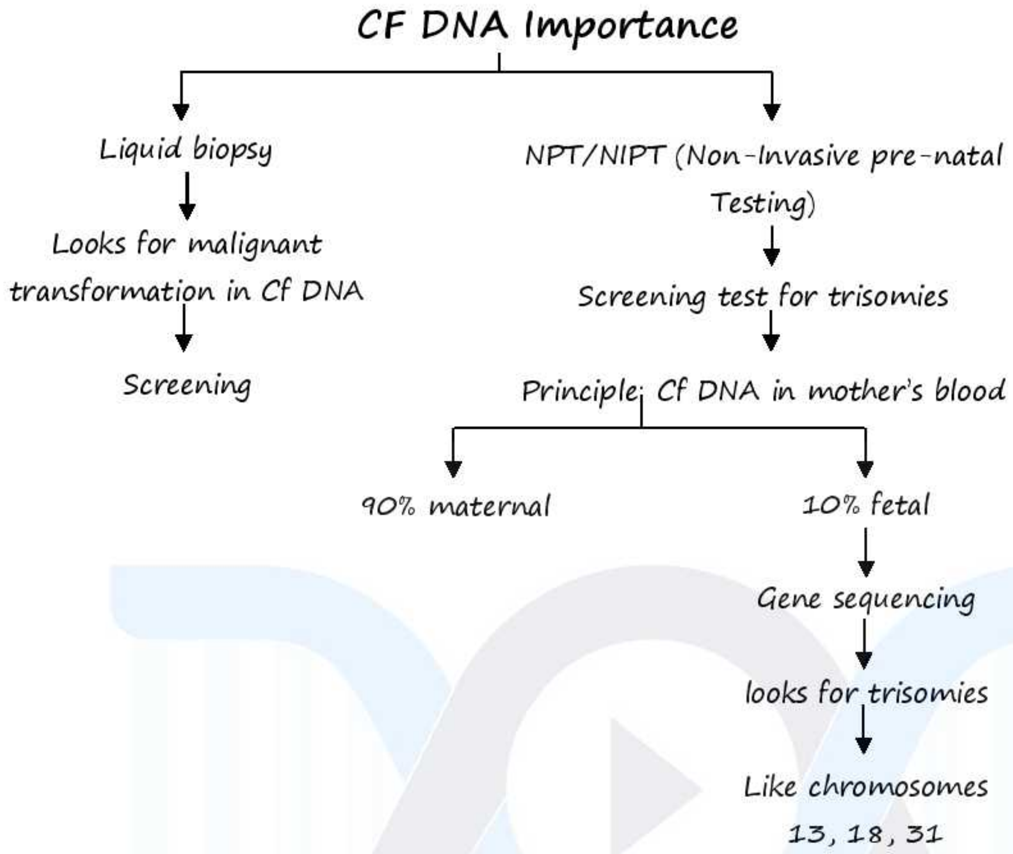
- Nuclear DNA
- Mitochondrial DNA
- Cf DNA (Cell free DNA) – Due to tissue turnover

Note:

- When cell dies
- Contents spill out into blood


Protein
DNA
Non-functional enzymes
CF DNA

CF DNA 00:02:14


CF DNA Importance
Liquid biopsy
Looks for malignant transformation in Cf DNA
Screening
NPT/NIPT (Non-Invasive pre-natal Testing)
Screening test for trisomies
Principle: Cf DNA in mother's blood
90% maternal
10% fetal
Gene sequencing
looks for trisomies
Like chromosomes 13, 18, 31

- Sensitivity of NIPT is >99%
- If NIPT is positive→ Follow up with confirmatory tests like amniocentesis or CVS (Chorionic villus sampling)

Nuclear DNA 00:10:32

- Most common form of nuclear DNA is B-form

Note:

- DNA in cell
  - i. Nuclear >99%
  - ii. Mitochondrial <1%

Properties of B-form DNA

- Double stranded

- Antiparallel
- Linear
- Double helical structure
- Right-handed/Clock wise
- Grooves
  i. Minor
  ii. Major → DNA – Protein interaction – regulation

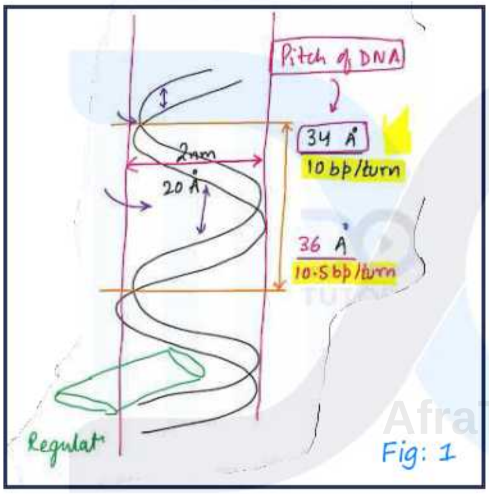

Pitch of DNA
34 Å
10 bp/turn
2nm
20 Å
36 Å
10.5 bp/turn
Regulatⁿ

Fig: 1

- Width of helix = 2nm/20A^o
- Pitch of DNA or distance occupied by 1 turn of helix = 34A^o (10 base pains/turn); = 36A^o (10.5 base pains/turn) – More accurate
- Minor Groove
- Major Groove

Note

- nm = 10^{-9}m
- A^o = 10^{-10}m
- μ = 10^{-6}m

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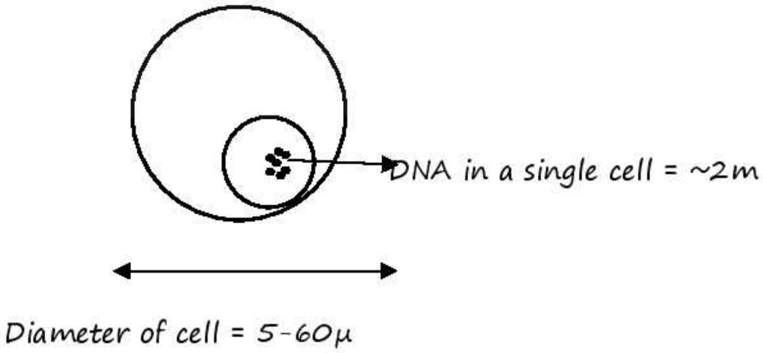
Z form DNA

- 2^{nd} most common form of DNA
- Double stranded
- Anti – parallel
- Linear
- Zig – Zag shape (hence the name Z form)
- Left-handed/anti-clockwise
- No grooves
- Pitch = 12 base pairs per turn (12bp/turn)
- A form of DNA
- 11 bp/turn
- C, D, E forms of DNA
- 8 bp/turn

Other Properties are similar to B form, only difference in dimensions

Organization of DNA - 00:20:59

- Nuclear DNA is highly organized (4 to 6 levels of organization)



Levels of organization	Shape
1st	Solenoid shape (nucleosome)
2nd	Super solenoid
3rd	Loops
4th	Whorls
5th	Solenoid
6th	Super solenoid (Chromatin)

Note:

- Chromatin and chromosome (More compact version)

  → Same level of organization

- Chromatin is seen in all stages of cell cycle except in M phase (Chromosomes → M phase)

Nucleosome

00:25:07

- Solenoid

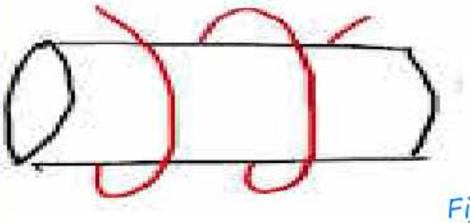


Fig:2

- Cylinder represents – histone octamer (8 sub units)
  - H2A x 2
  - H2B x 2

- H3 x 2
- H4 x 2

Note:

- H3 and H4 can form a dimer
- DNA wound around histone octamer
  - i. 146 base pairs
  - ii. 1.75 loops
- Multiple solenoids in continuation

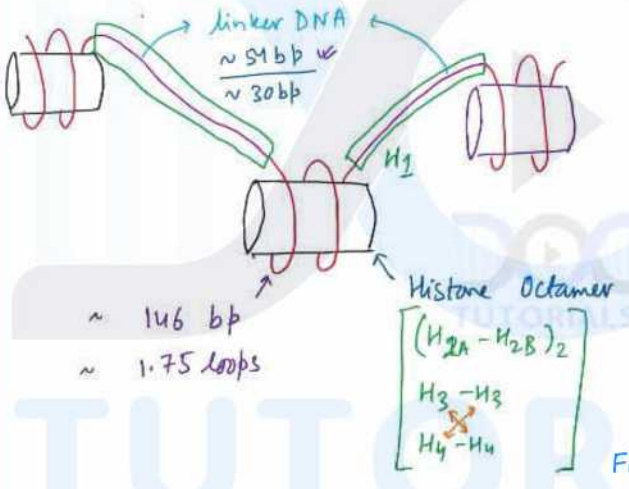

linker DNA
~ 54 bp
~ 30 bp
H1
Histone Octamer
(H2A - H2B)2
H3 - H3
H4 - H4
~ 146 bp
~ 1.75 loops

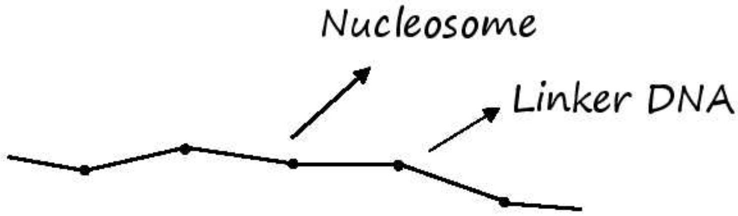
Fig: 3

Linker DNA:

- Not a part of nucleosome
- Link's nucleosomes
- Associated with H1
- Consists ~54 base pairs > ~30 base pairs

Note:

- Contrast electron microscope – detailed visualization of nucleosome
- Electron microscope – beads on string appearance



Cell death

Apoptosis
↓
Cleavage occurs within linker DNA, nucleosome structure not disrupted
↓
Result – multiples of nucleosomes
↓
On DNA gel electrophoresis ladder pattern

Necrosis
↓
DNA fragments of all sizes
↓
On DNA gel electrophoresis Smear pattern

Histones 00:33:43

- Basic proteins – due to basic amino acids (lysine, arginine)
- 5 different histones
  - H1
  - H2A
  - H2B
  - H3
  - H4

DNA – histones interaction:

Interact

DNA (Acidic) ⟷ Histones (Basic)

Due to -ve charge of phosphate

Due to basic amino acids

Types of covalent modification 00:35:37

Types of covalent modification

Histones
- Acetylation/Deacetylation
- Phosphorylation/Dephosphorylation
- ADP-Ribosylation/Removal
- Methylation/Demethylation
- Monoubiquitylation
- SUMOylation (SUMO = Small Ubiquitin Like Modifier)
- Citrullination

DNA
- DNA methylation

- Covalent modification is used in regulating rate of transcription and replication of DNA

Example:

- If phosphate added to histone
- Histone becomes less basic
- Strength of attraction between DNA and histone decreases
- Easy separation of DNA from histones
- Faster replication or transcription

Epigenetics 00:38:50

- Covalent modification of histones or DNA without changing the sequence of nucleotides

Impart some information related to replication and transcription

↓

Known as epigenetics

Information imparted by the sequence of Gene

↓

Known as genetics

Epigenetics is described as Gene – Environment bridge

Genotype

↓

Epigenetics

↓

Phenotype

- Epigenetics is transmissible or inheritable
- To identify epigenetics (especially DNA methylation) – special type of PCR

DNA – 2

Chromatin 00:00:18

- **Chromatin** (last level of organization of DNA in all phases of cell cycle – $G_0/G_1/S/G_2$)
- In 'M' phase → chromosome

$$\begin{tikzcd}
 & \text{Chromatin} \arrow[dl] \arrow[dr] & \\
\text{Euchromatin} & & \text{Heterochromatin}
\end{tikzcd}$$

Euchromatin	Heterochromatin
Transcriptionally active	Transcriptionally inactive
Uncondensed form of DNA	Condensed form of DNA
Lightly stains with dye	Darkly stains with dye

On staining DNA:

- Dye attaches to DNA at equidistant positions on both the strands

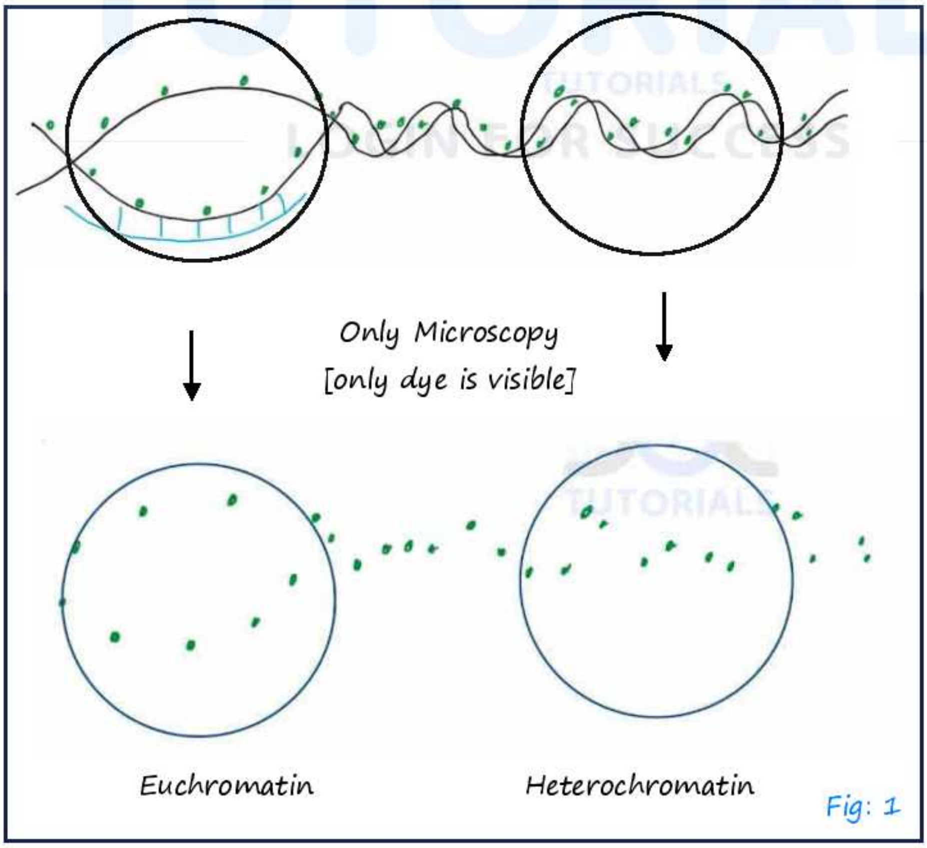
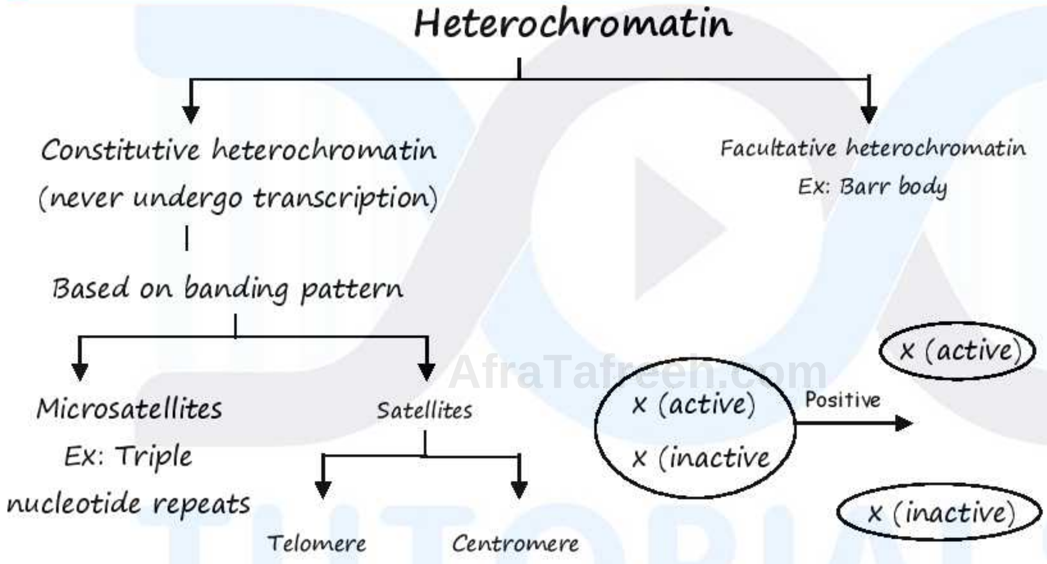

Only Microscopy
[only dye is visible]
Euchromatin
Heterochromatin

Fig: 1

Euchromatin 00:06:03

- Dynamic within a cell (i.e., euchromatin can become heterochromatin)
- Variable among different cells (i.e., the same DNA segment is euchromatin in one cell and heterochromatin in other cell)

Heterochromatin 00:08:33


Heterochromatin
Constitutive heterochromatin
(never undergo transcription)
Based on banding pattern
Microsatellites
Ex: Triple nucleotide repeats
Satellites
Telomere
Centromere
Facultative heterochromatin
Ex: Barr body
x (active)
x (inactive
Positive
x (active)
x (inactive)

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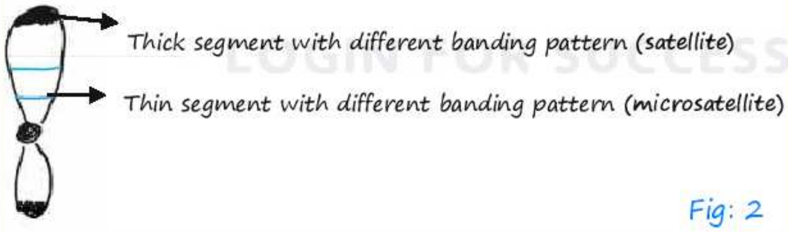

Thick segment with different banding pattern (satellite)
Thin segment with different banding pattern (microsatellite)

Fig: 2

Telomere 00:12:45

- Ends of DNA
- Sequence is self-complementary& repeating

↓

i.e., the strand pairs up with itself upon folding

- Functions

  i. Stabilizes the end of DNA

  ii. Protects from DNA repair machinery

end of DNA of a chromosome ↓ end of DNA of another chromosome

DNA repair machinery
recognizes as DNA break

↓

repair the damage

↓

2 ends of DNA are joined

↓

45 chromosomes
(Normal – 46 chromosomes)

↓

P53 (guardian of genome) recognizes loss of DNA

↓

Alarm

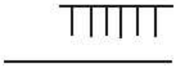
↓

Apoptosis of cell

DOC TUTORIALS LOGIN FOR SUCCESS

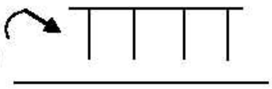
Role of telomere in protection from apoptosis:

- End of DNA



↓

telomere folds upon
itself as the sequence
is self-complementary



↓

Strain in the preceding part of DNA

↓

Some segments open up i.e. denaturation

↓

Upper strand – folded upon itself and small segment is denatured

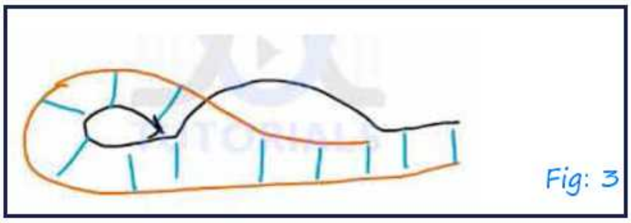


Fig: 3

Lower strand – folded upon itself and comes into the gap which is formed by denaturation

↓

The end of DNA is buried inside the DNA itself

↓

When two ends of DNA come together

↓

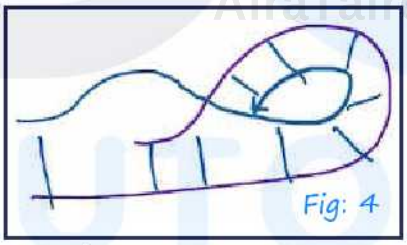


Fig: 4

Note:

Telomere deletion

↓

ends of DNA exposed

↓

apoptosis

↓

DNA repair mechanism cannot act as the ends are not visible

↓

No apoptosis of cell

In telomere: 00:21:26

- D loop
- Hogsteen base pairing and Chargaff base pairing
- Triple stranded DNA

Centromere: 00:22:31

- So called centre of chromosome/DNA
- Types of chromosome based on position of centromere

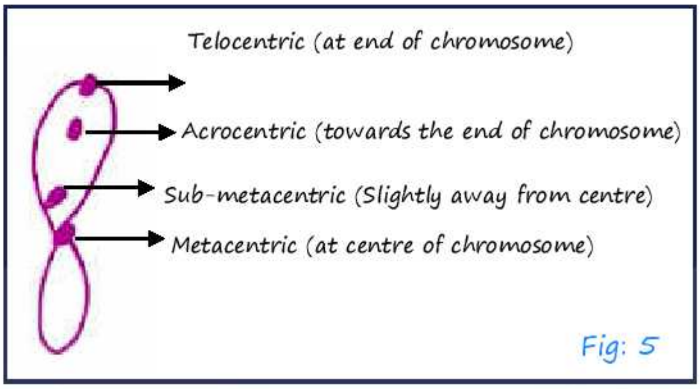

Telocentric (at end of chromosome)
Acrocentric (towards the end of chromosome)
Sub-metacentric (Slightly away from centre)
Metacentric (at centre of chromosome)

Fig: 5

- Repeating segments
- Function: provides docking site (point of attachment) for microtubules.

Note:

- Telomere and centromere are not associated with inherited genetic disorders because:

i. Constitutive heterochromatin (no transcription)

ii. No regularity function (i.e don't allow attachment of regulatory proteins)

Microsatellites 00:26:24

- 2 to 6 nucleotides repeating 'n' number of times $(2\text{-}6\ nt)_n$
- Example: $(3\ nt)_n$ → Triple nucleotide repeats

↓

Have regulatory function (allow attachment of regulatory proteins)

↓

Hence associated with inherited genetic disorder

- Triple nucleotide repeat disorders:

  Fragile x syndrome

  Huntington's disease

MITOCHONDRIAL DNA

Mitochondrial DNA – properties 00:01:03

1.Circular (no polarity)

2.Double stranded

Heavy strand/chain
most of the genes

Light strand/chain
very few genes

3.Small size (16,569 base pairs)

4.37 genes

37 genes

13 codes for proteins
↓
all belong to electron transport chain/respiratory chain

2 Code for rRNA

22 Code for tRNA

- Note: Total Electron Transport Chain (ETC)/Respiratory proteins

↓

67 proteins

↓

13 proteins (i.e., <20% of total proteins) come from mitochondria

5.No introns → simultaneous transcription and translation occurs

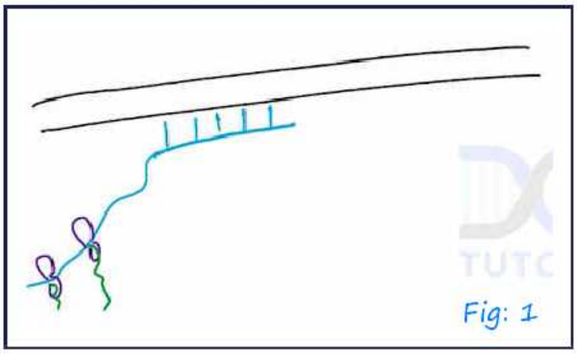


Fig: 1

6.Very few untranslated sequences

(In nuclear DNA – only 1.5% DNA is translated)

7.High mutation rate – as proof-reading activity is not efficient

(5 to 10 times more than nuclear DNA)

8.Exceptions to genetic code:

Codon	Normal Translation	Mitochondrial Translation
UGA	Stop	Tryptophan
AGA, AGG	Arginine	Stop Codon
AUG	Methionine	Formyl Methionine
AUA	Isoleucine	Methionine

- Note:

  - Stop codons
    - Nuclear DNA → 3
    - Mitochondrial DNA → 4

- 1st amino acid to be added
  - Prokaryotes → formyl methionine
  - Eukaryotes → methionine

- Q) Where do new mitochondria come from?
- A) From pre-existing mitochondria by binary fission

- Q) Source of mitochondria in zygote?
- A) Only from mother

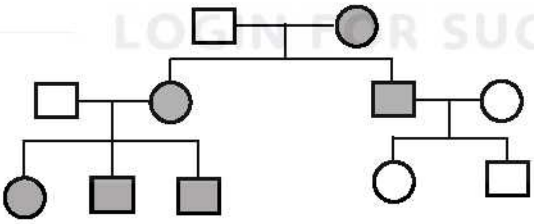
Inheritance Pattern of Mitochondrial DNA Disorders

00:13:15

Inheritance

- An affected female transmits disease to all offspring
- An affected male cannot transmit the disease

Pedigree chart:



Examples of Mitochondrial DNA Disorders:

00:16:21

1. Diabetes Milletus associated with deafness
2. LHON (Leber's Hereditary Optic Neuropathy)

3.Leigh syndrome (Subacute sclerosing encephalopathy)

4.NARP syndrome (Neuropathy, Ataxia, Retinitis Pigmentosa, Ptosis)

5.Myoclonic epilepsy with ragged red fibres- on muscle biopsy (MERRF)

6.Mitochondrial myopathy, Encephalopathy, Lactic acidosis, Stroke like symptoms (MELAS) – most commonly identified mitochondrial DNA disorder

7.Chronic Progressive External Ophthalmoplegia (CPEO)

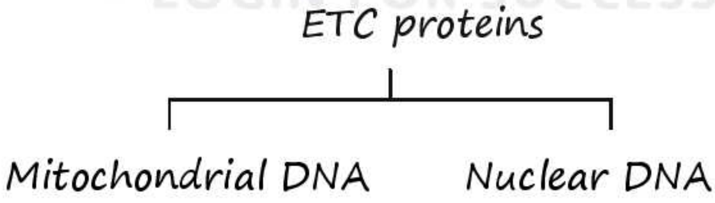
8.Kearns-sayre syndrome

9.Pearson syndrome

10. Infantile myopathy with lactic acidosis

Note

- Common feature in all mitochondrial DNA disorders → lactic acidosis → due to affected Electron Transport Chain (ETC)

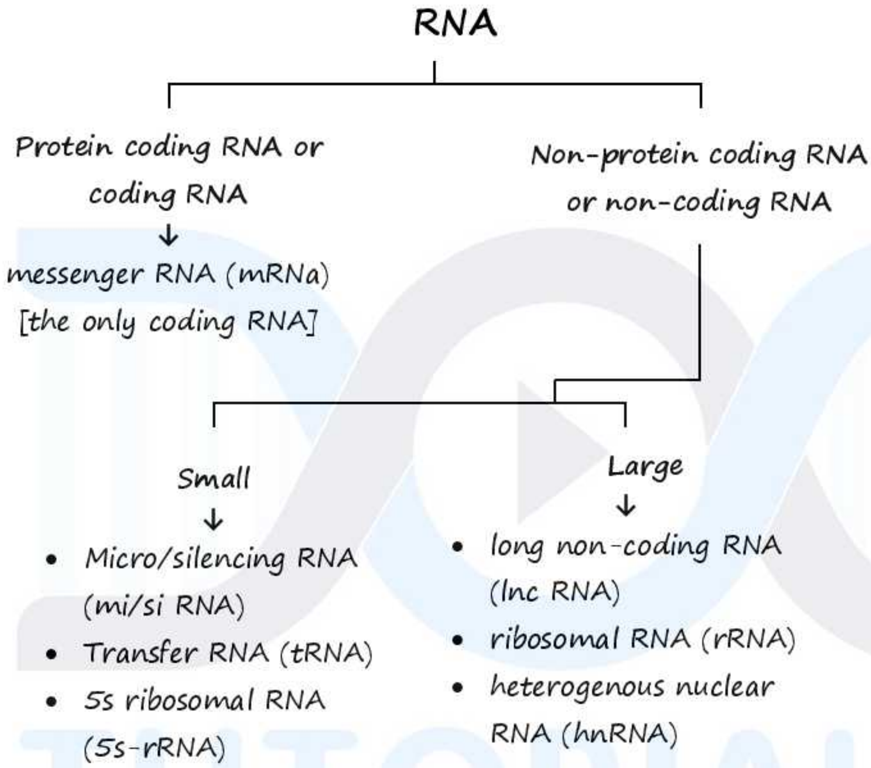


- So mitochondrial DNA disorders can also be due to nuclear DNA damage

RNA-1

RNA 00:00:17

- Ribonucleic acid (sugar-ribose)
- Polynucleotide

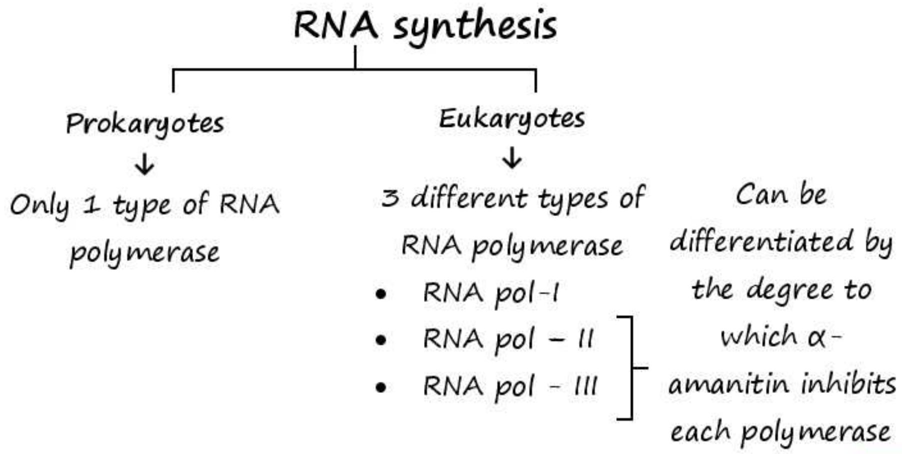


RNA

- Protein coding RNA or coding RNA
  ↓
  messenger RNA (mRNa) [the only coding RNA]
- Non-protein coding RNA or non-coding RNA
  - Small
    ↓
    - Micro/silencing RNA (mi/si RNA)
    - Transfer RNA (tRNA)
    - 5s ribosomal RNA (5s-rRNA)
  - Large
    ↓
    - long non-coding RNA (lnc RNA)
    - ribosomal RNA (rRNA)
    - heterogenous nuclear RNA (hnRNA)

- Note – Protein coding RNA: – mRNA > hnRNA (as mRNA comes from hnRNA)

RNA Synthesis: 00:04:58



RNA synthesis

- Prokaryotes
  ↓
  Only 1 type of RNA polymerase
- Eukaryotes
  ↓
  3 different types of RNA polymerase
  - RNA pol-I
  - RNA pol – II
  - RNA pol – III

  Can be differentiated by the degree to which α-amanitin inhibits each polymerase

- Most profound inhibition is for RNA pol – II

Type of RNA	Synthesized by
rRNA	RNA pol – I
hnRNA, snRNA, mi/si RNA	RNA pol – II
tRNA, 5srRNA	RNA pol – III

snRNA
Small nuclear
RNA

- RNA polymerase – II is associated with enzymes for capping and tailing, therefore RNA types synthesized by RNA pol – II have cap and tail.

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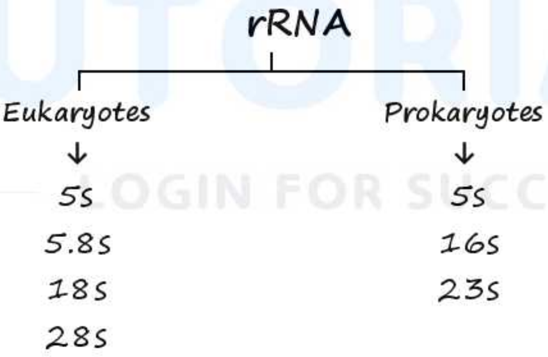
rRNA

00:09:23


rRNA
Eukaryotes
5s
5.8s
18s
28s
Prokaryotes
5s
16s
23s

rRNA	Synthesized by
5s	RNA pol – III
5.8s,18s,23s	RNA pol – I

- RNA polymerase – I → 45s rRNA

Post-transcriptional modification undergo processing and generate various segments → inside nucleus

18s 5.8s 28s

- S → Sedimentation coefficient/svedberg coefficient
- The more the value of S, less is the force required to settle the particle and vice-versa
- 23s (prokaryotes) and 28s (eukaryotes)

↓

Perform catalytic function as peptidyl transferase during translation

↓

Hence known as ribozyme (RNA with catalytic activity)

- Other ribozymes – snRNA, intron

↓ ↓

splicing self-splicing activity

hnRNA 00:15:41

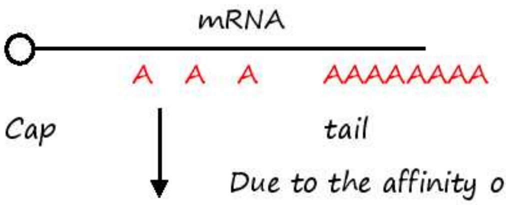
hnRNA $\xrightarrow{\text{Post transcriptional modification}}$ mRNA

- Changes in post-transcriptional modification: occur simultaneously

  1. Capping – addition 7-methyl guanosine at 5^{1}end
  2. Tailing – addition of poly adenine at 3^{1}end
  3. Splicing – removal of introns followed by joining of exons

Role of capping and tailing

- Increasing the half-life of mRNA



Due to the affinity of cap for Adenine (A) residues

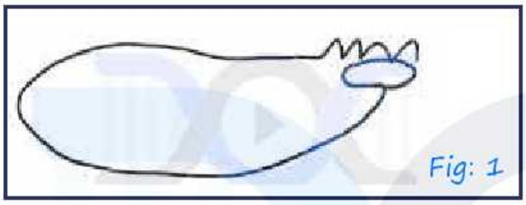


Fig: 1

Closed Structure

↓

Exonucleases fail to act as there is no end

↓

now damage of mRNA is carried by endonucleases

Which are less in amount compared to exonucleases

↓

Therefore, half life of mRNA increases

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- Capping – helps in identification of mRNA during translation

Splicing 00:20:28

- Mediated by snRNA
- snRNA – rich in uracil – so symbol for snRNA is U
- U_1, U_2, U_4, U_5, U_6 → splicing

- U_7 → Modification of histone mRNA
  - (histone mRNA do not undergo splicing, as there are no introns)
  - (The only eukaryotic genes without introns – histone genes)
- U_3 → not active in splicing
- snRNA
  +
  hnRNA — Spliceosome
  +
  Multiple proteins
- Introns are released as lariate → degraded by endonucleases and exonucleases
- Splicing is variable – multiple different mRNA obtained from same hnRNA

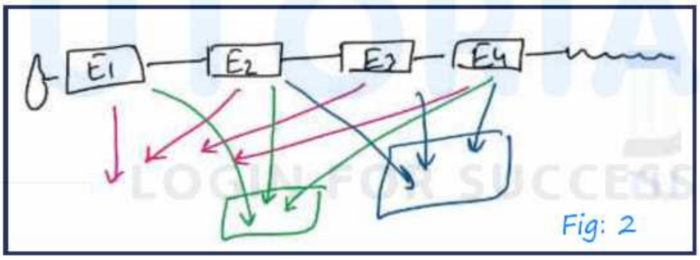


Fig: 2

Ways to obtain different mRNA:

- Selective splicing
- Alternative donor/upstream site
- Alternative acceptor/downstream site
- Alternative/variable poly A tail

Note:

- Due to post transcriptional modification i.e

  Variation in splicing

  ↓

  From 18,000 to 21,000 genes

  ↓

  >1 million proteins

RNA–II

Synthesis of miRNA 00:01:13

- DNA

↓ Transcription (RNA pol – II)

Primary micro-RNA
(pri-mi-RNA)

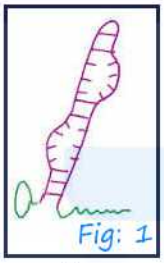


Fig: 1

- Stem loop structure
- Complementarity in some parts, non-complementary in some parts

↓ DROSHA
DGCR-8/Pasha

[Enzymes remove cap and tail]

Pre micro RNA
(pre-mi-RNA)

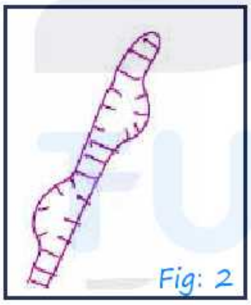
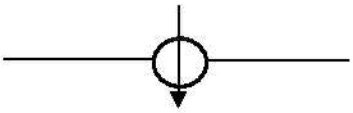


Fig: 2

Exportin-5 – pushes pre-mi-RNA into cytoplasm via nuclear pore

Nucleus



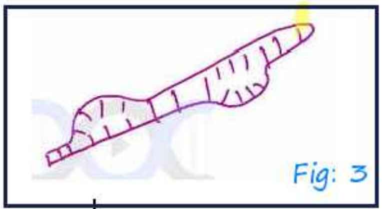


Fig: 3

↓

Dicer (removes the loop of pre-mi-RNA)

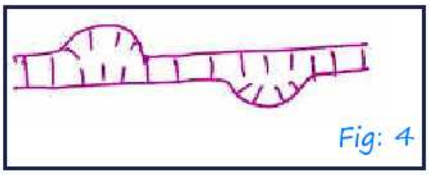


Fig: 4

mi RNA duplex

↓

2 strands separate

↓

One of the strands enter into RISC
(RNA Induced Silencing Complex)

RISC size of mi-RNA is RISC
– 18 to 26 nucleotides

↓

RISC activates

↓

act on mRNA in the cytoplasm

Cytoplasm

- Note:
  - Post transcriptional modification can occur in
    miRNA
    rRNA
    hnRNa

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Action of miRNA 00:11:07

- Regulation of gene expression (1 miRNA regulates 50 to 100 genes)
- Regulation occurs by RNA interference (RNAi):
- Discovery of RNAi → Nobel prize in 2006
- Mechanism of RNA: - occurs in cytoplasm
  - Degradation of mRNA – occur only when complete complementarity is there between miRNA and mRNA

- Destabilization of mRNA
- Prevent translation of mRNA

Partial complementarity is enough

Result of RNA:

- Knock down phenomenon
  - Occurs at RNA level in cytoplasm
  - Total number of protein copies from mRNA decreases

Note

- Knock out (deleting a part of gene)
- knock in (Introducing a foreign gene)

Occur at DNA/gene level

- miRNA
  - Therapeutic tool against RNA viruses
  - Large side effect profile

Silencing RNA/siRNA 00:17:09

- In plants, similar structure as miRNA
- RNA: with siRNA – occurs only when complete match i.e complementarity is present

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Transfer RNA/tRNA 00:18:20

- Also known as adapter molecule (because it converts the sequence of codon into the sequence of amino acids)
- Shape

  i. 2D → cover leaf

  ii. 3D → inverted 'L' shape

Arms of tRNA:

- Variable arm:

  - No known biological function
  - Based on length of variable arm

    1.3 to 5 nucleotides long →Type 1 tRNA

    2.>10 nucleotides long →Type 2 tRNA

- TΨC arm:
  - Attaches tRNA to ribosomal surface
  - Ψ – Pseudouridine
  - T – Thymine

  result of post-transcriptional modification (Modified nucleotides)

- Acceptor arm:
  - Amino acids attach at 3^1 end of acceptor arm
  - At 3^1end: 5^1-CCA-3^1 (seen in all tRNA at 3^1 end)

    ↓

    Due to post-transcriptional modification

    ↓

    Nucleotides are freshly added, not modified
  - Amino acids attach by **amino acyl tRNA synthetase** (enzyme)
  - This enzyme occur in multiple variants for every different amino acid
  - 2 ATP required – every time when a amino acid is attached

- D arm:
  - Also known as determining arm because it selects the correct variant of the enzyme which further attaches the correct amino acid on acceptor arm
  - Contains dihydrouridine – modified nucleotide **(example of post transcriptional modification)**

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Anti-codon arm:

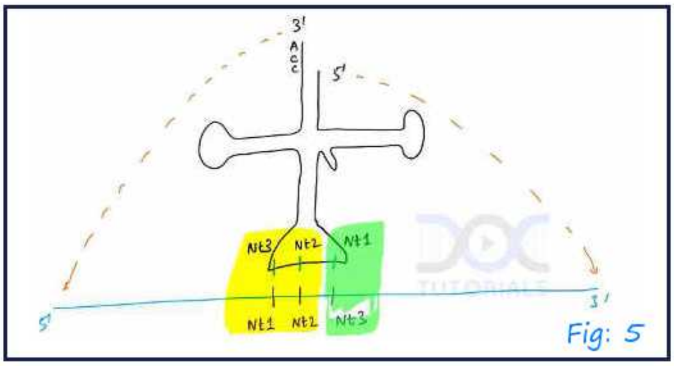


Fig: 5

- tRNA and mRNA are anti-parallel
- Nucleotides are read from 5^1-3^1

Interaction between

Nucelotide 1 and 2 (codon)
⊕
Nucleotide 3 and 2 (anti-codon)
↓
Strictly as per Chargaff's rule

Nucelotide 3 (codon)
⊕
Nucleotide 1 (anti-codon)
↓
Wobble phenomenon

- Wobble phenomenon: The same nucleotide on tRNA i.e Nucleotide 1 is able to pair with 2 different types of Nucleotides at Nucleotide 3 of codon
- Wobble site: Nucleotide 1 of anti-codon
  - Sometimes wobble site may have hypoxanthine as nitrogenous base due to post transcriptional modification (modified nucleotide from Adenine)
  - Wobble phenomenon and site are partly responsible for silent mutation

Arms showing post-transcriptional modification:

1. TΨC
2. Acceptor arm
3. D-arm
4. Anti-codon arm

Modified nucleotides in tRNA:

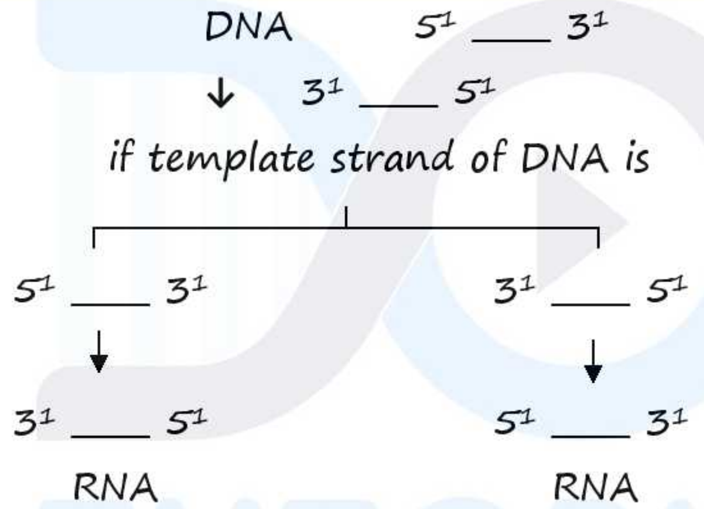
Modified nucleotide	Arm	Modified from
Thymine	TΨC arm	Uracil
Pseudouridine	TΨC arm	Uracil
Dihydrouridine	D arm	Uracil
Hypoxanthine	Anti-Codon arm	Adenine

DNA-RNA SEQUENCE RELATIONSHIP

DNA 00:00:55

- Double stranded
- Strands are anti-parallel 5^1 ___ 3^1
  3^1 ___ 5^1
- Genes can be present on any strand

Transcription 00:01:25


DNA
5^1 ___ 3^1
3^1 ___ 5^1
if template strand of DNA is
5^1 ___ 3^1
3^1 ___ 5^1
3^1 ___ 5^1
5^1 ___ 3^1
RNA
RNA

- RNA is formed by adding complementary nucleotides by chargaff's rule
- DNA → T and RNA → U

Note: -

- Whenever two nucleic acids interact, their alignment will be anti-parallel
- The direction of synthesis of any nucleic acid is always 5^1 ___ 3^1

DNA strands in comparison to RNA 00:02:57

DNA

Template/Non-coding/
Anti-sense strand
↓
Anti-parallel orientation and
complementary to RNA

Non-Template/coding/
Sense strand
↓
Same orientation and same
coding sequence as RNA

Example 00:05:57

To de-code sequence of mRNA

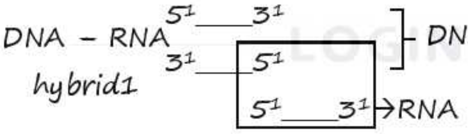
If coding strand of DNA is given
↓
Replace 'T' by 'U'

5^1 C A G T T C G 3^1
5^1 C A G U U C G 3^1

If template strand of DNA is given
↓
Change the orientation
Write complementary bases
Replace 'T' by 'U'

5^1 C A G T T C G 3^1
5^1 G U C A A G C 5^1

DNA-RNS hybrid: 00:11:29



- Question: what is the sequence of RNA, if the sequence of DNA in a DNA-RNA hybrid is given?
- Answer: As RNA is formed from template strand, in DNA-RNA hybrid the given DNA sequence can be considered as template strand

GENETIC CODE

Genetic code 00:00:11

- Translation of codons into amino acids.

Properties of Genetic Code 00:01:10

1. **Genetic code is triplet** (3 nucleotides code for one amino acid)

4 x 4 x 4	
A	AA
G	GG
C	CC
U	UU

→ 64 condons (based on permutation and combination)

→ 3 condons – stop condons

→ 61 condons – code for 20 amino acids

- 4 Possibilities of nucleotides
- If 5 nucleotides → 125 condons
- If 3 nucleotides → 27 condons
- If genetic code is
  - doublet code _ X _
  - Quadruplet code _ X _ X _ X _ X _

2. Degeneracy of genetic code/Degenerate property:
   - Multiple codons code for same aminoacids
   - One of the reasons for silent mutation
3. Unambiguous:
   - Translation of codon will always code for same amino acid
4. Non-overlaping
   - One nucleotide can be used for only one codon.

$5^1\ \overbrace{\text{AUG}}\underbrace{\text{CAG}}\ 3^1$

5.Non-punctuated/commaless:

- No nucleotide can be skipped

$5^1\ \underbrace{\text{AUG}}\ \text{C}\ \underbrace{\text{AGG}}\ \text{CC}\ 3^1$

6.Universal

- Same translation for codons in all organisms (some exceptions)
- Indicates common progenitor cell during evolution

MUTATIONS

Mutation vs Polymorphism 00:00:45

- Variation in the sequence of nucleotides in a gene
- Same definition is for polymorphism
- The difference is statistical
- If % of population showing the change in nucleotide sequence is

<1% population
↓
Mutation

>1% population
↓
Polymorphism

Polymorphism

- First mutation occurs

↓

Gradually spreads into population without affecting life

↓

Establishes into polymorphism

- All polymorphisms come from mutations
- So the commonest mutation (i.e. point mutation) is the commonest cause for polymorphism
- Most common type of polymorphism – SNP (Single nucleotide polymorphism)
- Source of SNP is the most common type of mutation i.e., Point mutation

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- SNP is commonly used for RFLP studies (Restriction fragment length polymorphism)

Types of Mutation 00:03:17

i. Base substitution/Point mutation – **most common type**

ii. Insertion/Deletion –**2nd most common type**

Point mutation 00:03:51

Point mutation

- Transition
  - Purine replaced by purine
  - (or) A ⇄ G
  - Pyrimidine replaced by pyrimidine
  - C ⇄ U
- Transversion
  - Purine replaced by pyrimidine
  - A⇄C or G ⇄ C
  - A ⇄ U G ⇄ U
  - Pyrimidine replaced by pyrimidine
  - U ⇄A C ⇄A
  - U⇄G C ⇄G

Effect of Point Mutation with Example

- A G A (codon) → Arginine

Point mutation	Amino acid	Type of mutation	Effect on protein
A G G	Arginine	Silent mutation (due to degeneracy of genetic code)	Same protein
G G A	Glycine	Missense mutation	Same size of protein but one amino acid is changed
U G A	Stop codon	Non-sense mutation	Truncated/small protein

Missense mutation (MSM)

Acceptable MSM	Partially acceptable MSM	Unacceptable MSM
↓	↓	↓
No change in physical chemical and biological properties of protein	Change in physical and/or chemical properties but biological activity is unchanged	Loss of biological activity
Example: Hb Hikari, Hb Adelaide, Hb Milwaukee,	**Example:** HbS, HbC HbD HbE, HbO	**Example:** Inherited methemoglobin or HbM or Hb Boston

HbS

- Most common abnormal variant of Hb
  - In β chain of Hb

    ↓

    In A – helix

    ↓

    At 6th position of peptide chain

    ↓

    Normal glutamic acid is replaced by valine

    (mnemonic: G-gone)
  - Glutamic acid – negative charge – repel RBCs
  - Valine – neutral – RBCs stick together → sickling

Insertion and Deletion Mutation 00:19:14

- Frame shift mutation
- Example: 5^1 AUG CCA GCA 3^1

Insertion → 5^1 AUG ACC AGC 3^1 – reading frame shift to left

Deletion → 5^1 AUG CAG CA 3^1 – reading frame shift to right

Types of insertion and deletion mutation:

a. Pair of insertion – deletion:

Pair of insertion – deletion

An insertion followed by deletion

An insertion followed by insertion

Effect

Temporary/Transient frame shift mutation

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- Example: AUG CCA GCA CUG

A↓ ↗ ↘

AUG ACC AGC CUG

b. Insertion/Deletion in multiples of 3:

- Effect
  - Insertion – gain of amino acid
  - Deletion – loss of amino acid
- No frame shift mutation

PURINE SYNTHESIS

- Dietary purines and pyrimidines are not absorbed
- No storage molecule for purines & pyrimidine

Purine synthesis

- De-novo synthesis
- Salvage pathway

De-novo Synthesis 00:00:15

1. Occur in all tissues
   - **Exception:** decreased in CNS (due to decreased enzyme 2)
   - absent in PMN, RBC (due to absence of enzyme 2)
2. Occur in cytoplasm
3. Substrate → Ribose-5-phosphate (source is HMP pathway)

HMP shunt

- Upregulated (ex: Von-Gierke disease) ↓ Increases Purine synthesis ↓ Increases uric acid production
- Downregulated ↓ Decreases Purine synthesis ↓ Decreases uric acid production

- Ribose-5-phosphate → do not contribute to the ring in purine
- Donor Molecules to Purine ring

Donor	Donates	Donor to
Aspartate	1N	N_1
Glutamine	2N	N_3, N_9
Glycine	1N, 2C	C_5, C_4, N_7
Respiratory CO_2	1C	C_6
Formyl Tetra Hydro Folate	1C	C_2
Methenyl Tetra hydro folate	1C	C_8
Total:	4N, 5C → 9 atoms	

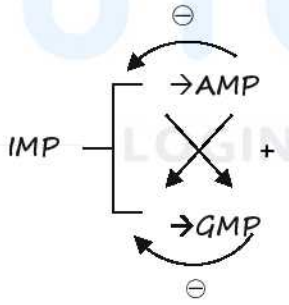
4. End product: Inosine mono phosphate (IMP) via two AMP or GMP reactions+

5.Rate limiting enzyme (most important): Enzyme 2

6.Target for regulation: Enzyme 1 (PRPP synthetase)

7.Enzyme 2 (PRPP Glutamyl Amido Transferase)

- Inhibited by AMP and GMP



- feedback inhibition of AMP, GMP synthesis from IMP
- AMP promotes the synthesis of GMP and GMP promotes the synthesis of AMP from IMP

8.Costly pathway (requires more ATP, as it is a long pathway)

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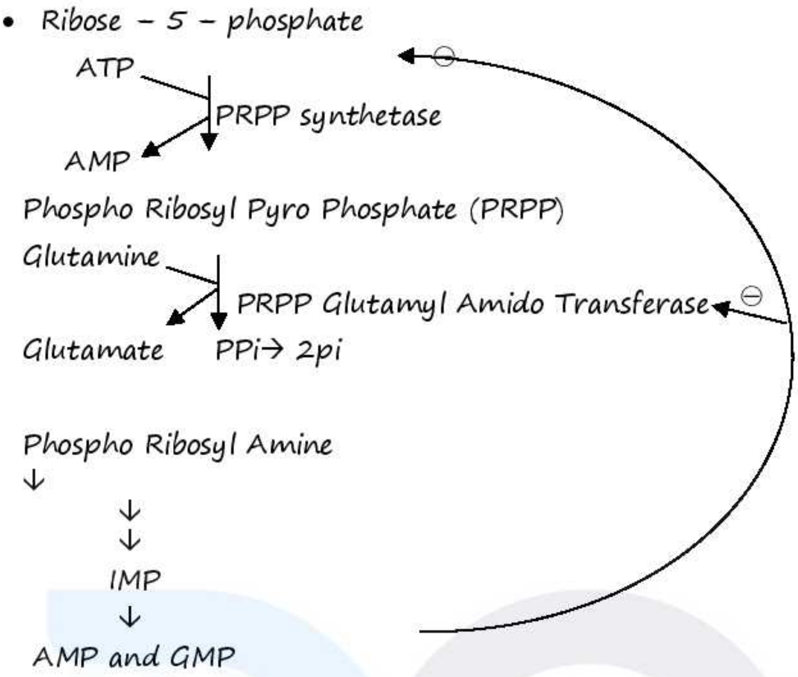
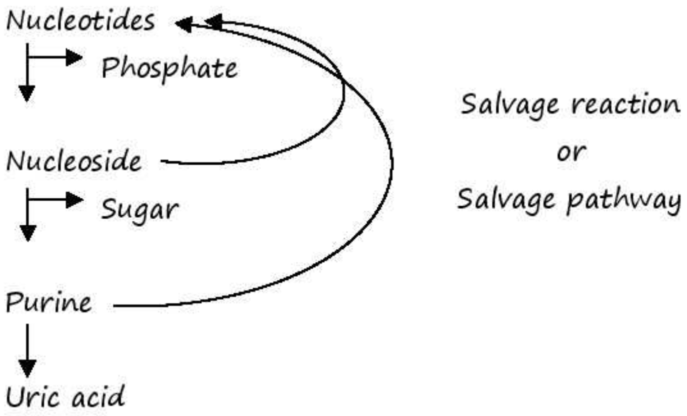
- Pathway


Ribose – 5 – phosphate
ATP
PRPP synthetase
AMP
Phospho Ribosyl Pyro Phosphate (PRPP)
Glutamine
PRPP Glutamyl Amido Transferase
⊖
⊖
Glutamate
PPi→ 2pi
Phospho Ribosyl Amine
IMP
AMP and GMP

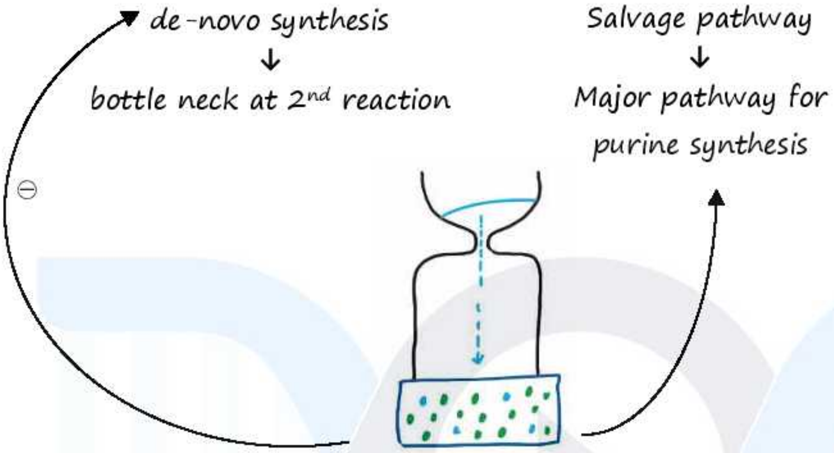
- Free purine is not synthesized (because the sugar and phosphate in the substrate attaches to the purine and forms nucleotide)
- 1st nucleotide to be synthesized → IMP
- 2 times cyclisation occurs during synthesis (because purine has 2 rings)
- Source of nucleotide for RBC (red blood cell) and for PMN (polymorphonuclear neutrophils) → borrow nucleotides from liver.

Salvage Pathway: 00:19:37


Nucleotides
Phosphate
Nucleoside
Sugar
Purine
Uric acid
Salvage reaction
or
Salvage pathway

- Minor pathway of purine synthesis for all tissues, except CNS
- In CNS

In CNS


de-novo synthesis
↓
bottle neck at 2nd reaction
⊖
Salvage pathway
↓
Major pathway for purine synthesis

Nucleotide pool required

- Synthesized nucleotides have feedback inhibition, there by a level of equilibrium is maintained for de-novo synthesis.
- Purines that participate in salvage pathway:

- $\text{Adenine} \xrightarrow{\text{PRPP} \quad \text{PP}} \text{AMP}$
APRTase

- $\text{Hypoxanthine} \xrightarrow{\text{PRPP} \quad \text{PPi}} \text{IMP}$
HGPRTase

- $\text{Guamine} \xrightarrow{\text{PRPP} \quad \text{PPi}} \text{GMP}$
HGPRTase

  - IF APRTase (Adenine Phospo Ribosyl Transferase) defective, it is not significant IMP forms sufficient AMP via 2 reactions

- IF HGRTase (Hypoxanthine Guanine Phospho Ribosyl Transferase) defective
  ↓
  IMP, GMP not synthesized
  ↓
  CNS affected
  (because salvage pathway is major pathway in CNS)
  ↓
  Lesch Nyhan Syndrome

Mechanism of hyperuricemia in Lesch Nyhan Syndrome 00:26:37

Salvage pathway blocked
↓
Purine nucleotides not formed
↓
Feedback inhinition to de-novo synthesis is lost
↓
more production of PRPP
↓
PRPP enters other tissues
↓
excess purine production
↓
excess uric acid production

- Primary cause of hyperuricemia in Lesch Nyhan Syndrome – Increased PRPP synthesis in CNS

TUTORIALS LOGIN FOR SUCCESS

LESCH NYHAN SYNDROME

Lesch Nyhan Syndrome 00:00:12

- Associated with salvage pathway
- Absence of HGPRTase
- Inheritance: x linked recessive – mostly males affected

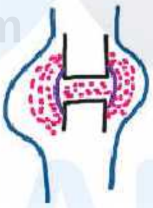
Biochemical presentation 00:01:59

Increases uric acid

Gout
- Painful joints

Nephrolithiasis
- Decreased/absence of urination
- Radiating pain

Tophi
- Bulbous stony/bony hard swelling around joints



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Neurological Presentation 00:05:35

- Mental retardation (poor performance)
- Dystonia (abnormal movements, frequent falls)

Neuropsychiatric presentation 00:06:10

- Very aggressive (frequent quarrelling with sibling, suspension from school)
- Self-mutilation tendency – muscle loss
  - Biting of lips, fingers

  - Bite marks on hands/feet
- Treatment: Pharmacological and behavioural therapy

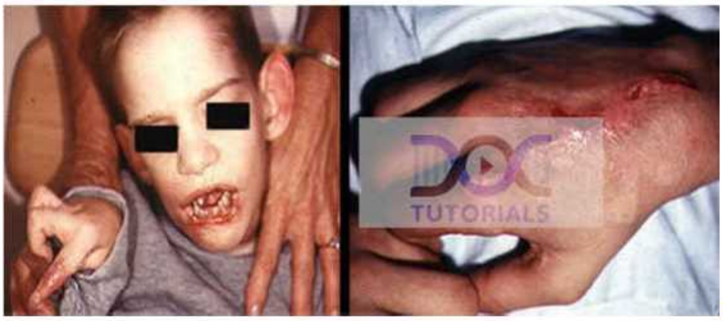


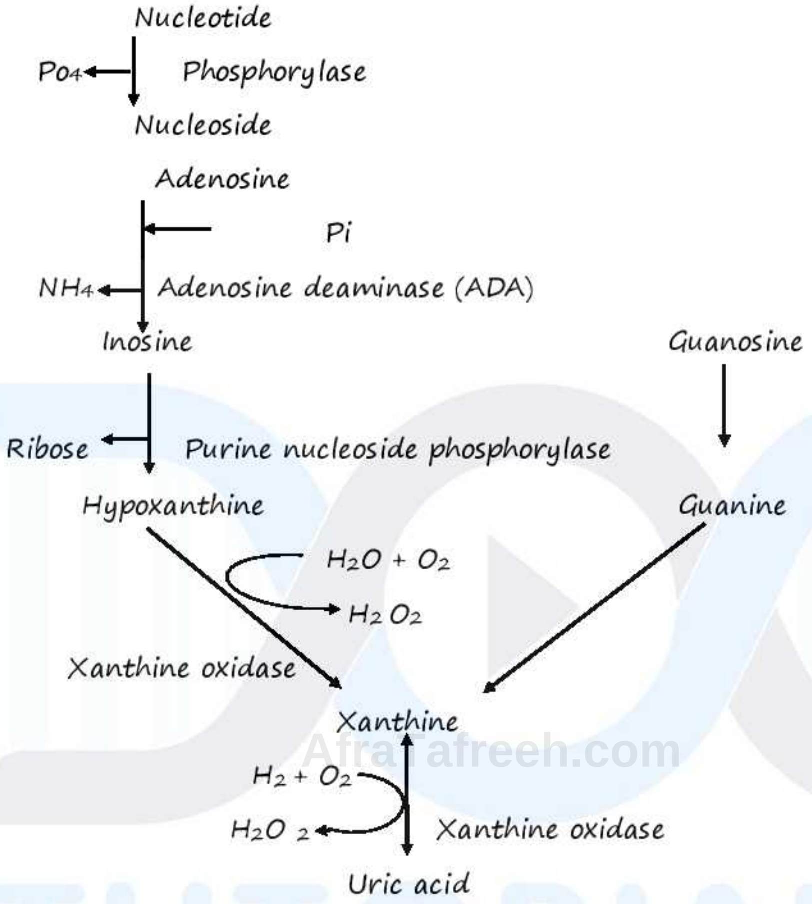
Fig: 1

Lesch Nyhan Variant 00:11:23

- Decreased amount of HGPRTase
- Also known as Kelley Seegmiller Syndrome
- No or low neurological and neuropsychiatric signs and symptoms (due to less uric acid formation comparatively)

PURINE DEGRADATION

Purine degradation pathway 00:00:44


Nucleotide
PO_4
Phosphorylase
Nucleoside
Adenosine
Pi
NH_4
Adenosine deaminase (ADA)
Inosine
Guanosine
Ribose
Purine nucleoside phosphorylase
Hypoxanthine
Guanine
$H_2O + O_2$
H_2O_2
Xanthine oxidase
Xanthine
$H_2 + O_2$
H_2O_2
Xanthine oxidase
Uric acid

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- Uric acid via backward reaction (i.e., uric acid → Xanthine) neutralizes H_2O_2 (Hydrogen peroxide), hence acting as anti-oxidant
- Xanthine oxidase – Differential activity in different individuals

Xanthine oxidase
- ↕ Activity genetically decreased→ Hypouricemia
- ↕ Activity genetically increased→ Hyperuricemia

PRPP synthetase
- ↕Activity decreased → Hypouricemia
- ↕Activity increased → Hyperuricemia

Disorders associated with purine degradation: ADA deficiency 00:06:42

- No breakdown of purines
- Accumulation of purine nucleotides (AMP, ADP, ATP)
- Feedback effect
- Resulting ADA Subtype of SCID (Severe combined immunodeficiency) – T and B cells formation affected

Nezelof's syndrome 00:07:10

- Absence of purine nucleoside phosphorylase
- ↓ Activity of T and B cells

Gout 00:07:43

- Uric acid – final degradation product in primate mammals
- Sparingly soluble, so sudden change in concentration/↓PH/↓Temperature → Uric acid gets precipitated
- Allantoin (Water soluble)
- Uricase – (in some animals) → rasburicase (treatment of gout)

TUTORIALS LOGIN FOR SUCCESS

Increased Uric Acid

↓

Gout

- Metabolic gout (Due to ↑ production of uric acid)
  ↓
  Metabolic causes
  - **Inherited** (primary metabolic gout)
    - Increases PRPP synthetase activity
    - Increases xanthine oxidase activity
    - von-Gierke disease
    - Hereditary fructose intolerance
    - Lesch Nyhan Syndrome/Variant
  - **Acquired** (secondary metabolic gout)
    - Increases tissue turnover
      - Malignancy
      - Myeloproliferative syndromes
      - psoriasis
    - Dietary causes
      - Liver rich meat
      - Beer
      - Excessive fructose
- Renal gout (Due to ↓ excretion of uric acid)
  - CKD/ESRD
  - NSAIDS (Kidney's damage)
  - Thiazide diuretics (Interface with UA execration)
  - Lactic acidosis (LA competes with UA for excretion)
  - Lead (Block the pump for UA excretion; saturnine gout – gout due to lead)

  Causes:
  - Severe exercise
  - Acute alcohol intoxication
  - Pregnancy

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Increased uric acid

Von Gierke disease:

Deficiency of Glucose-6-phosphatase

↓

HMP shunt upregulated

↓

More production of purines

↓

More degradation of purines

Hereditary Fructose intolerance:

Deficiency of aldolase – b

↓

Accumulation of phosphate

↓

More production of nucleotides

↓

More degradation

Diet:

Excess consumption of purines in diet

↓

Purine degradation end product (i.e. uric acid) reaches blood

Excessive fructose consumption:

Flooding of glycolytic pathway

↓

Diverts available glucose to HMP shunt

↓

HMP shunt upregulated

TUTORIALS LOGIN FOR SUCCESS

Lesch Nyhan syndrome/Variant:

Absence/deficiency of HGPRTase

Excessive beer consumption:

Beer (undistilled alcoholic beverage)

↓

Contains large amounts of yeast and bacteria required for fermentation

↓

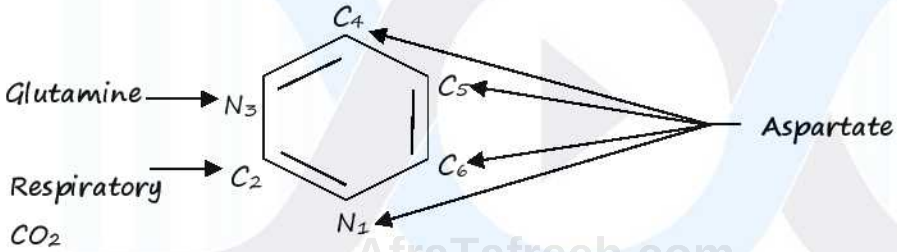
So content of genetic material i.e. nucleotides is very high

PYRIMIDINE METABOLISM

Pyrimidine synthesis

- Occur in all tissues (Maximum synthesis of purines and pyrimidines occur in liver)
- In cytoplasm
- Substrates and Donors
  - Glutamine: 1N
  - Aspartate: 1N, 3C
  - Respiratory CO_2:1C

Total: 2N, 4C→6 atoms



Pyrimidine ring

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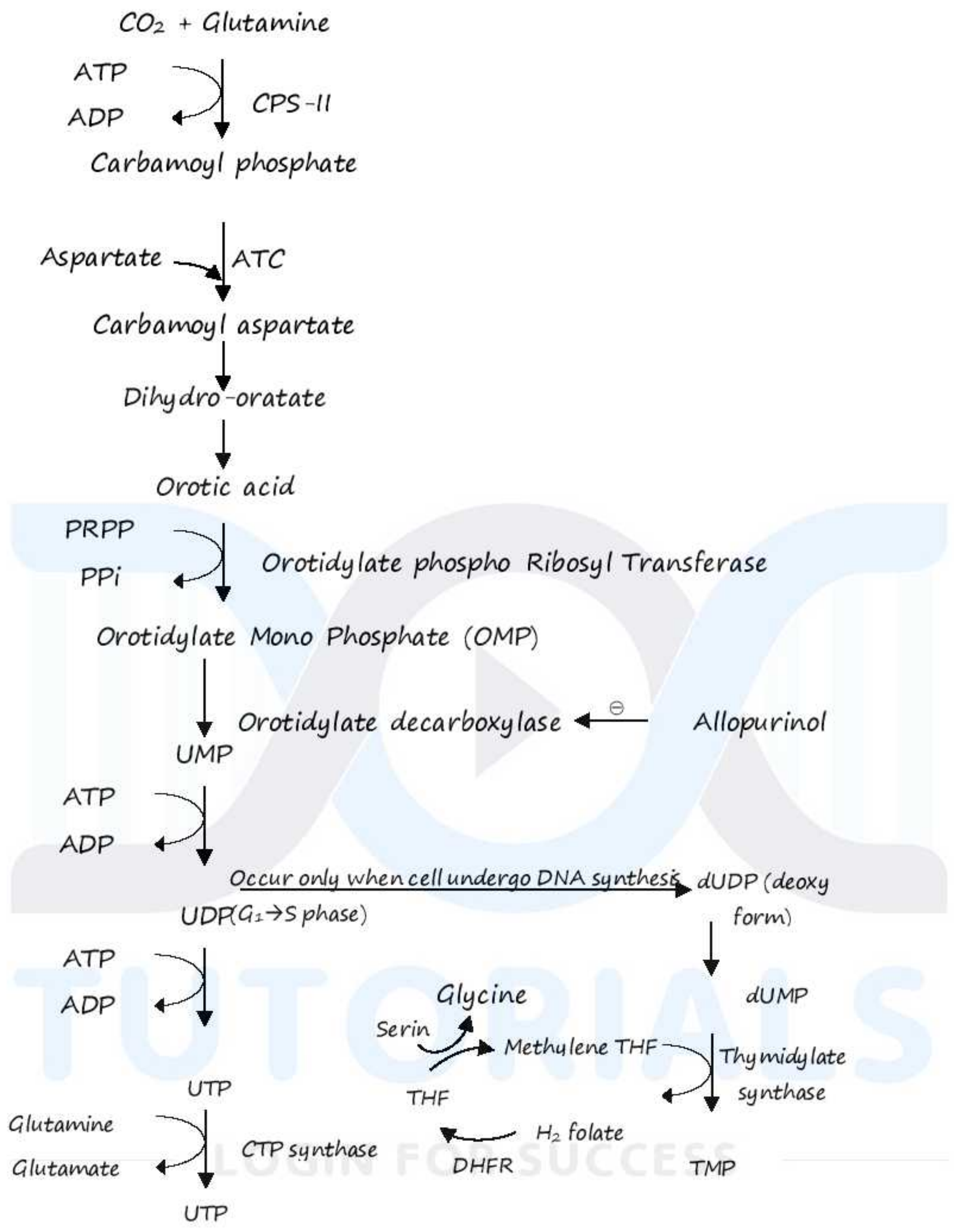
- End products: UTP, CTP, TMP/TTP
- Rate limiting enzyme (most important): enzyme 1
- Target for regulation
  - Enzyme 1 (Carbamoyl phosphate synthetase-II/CPS-II)
  - Enzyme 2 (Aspartate Trans Carbamoylase/ATC)
- Mechanism of regulation:

Enzyme 1
⊕ PRPP
⊖ UTP, Purine nucleotides

Enzyme 2
⊕ ATP
⊖ CTP

- Costly pathway

Pathway 00:08:10


CO_2 + Glutamine
ATP
ADP
CPS-II
Carbamoyl phosphate
Aspartate
ATC
Carbamoyl aspartate
Dihydro-oratate
Orotic acid
PRPP
PPi
Orotidylate phospho Ribosyl Transferase
Orotidylate Mono Phosphate (OMP)
Orotidylate decarboxylase
⊖
Allopurinol
UMP
ATP
ADP
Occur only when cell undergo DNA synthesis
UDP(G_1→S phase)
dUDP (deoxy form)
ATP
ADP
Glycine
Serin
Methylene THF
THF
dUMP
Thymidylate synthase
UTP
Glutamine
Glutamate
CTP synthase
H_2 folate
DHFR
TMP
UTP

- 1^{st} pyrimidine nucleotide to be synthesized is OMP
- Free pyrimidine i.e., orotic acid is synthesized
- TMP always have deoxy-ribose sugar

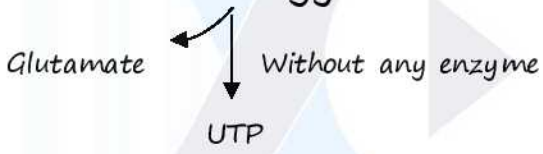
Chemotherapy (block DNA synthesis)

- 5-Fluorouracil ↓ Inhibits Thymidylate synthase
- Methotrexate ↓ Inhibits DHFR (dihydrofolate reductase) by affecting the co-enzyme

- Aminopterin – also inhibits DHFR
  - But weak inhibition, so not used in chemotherapy

Spontaneous deamination 00:17:37

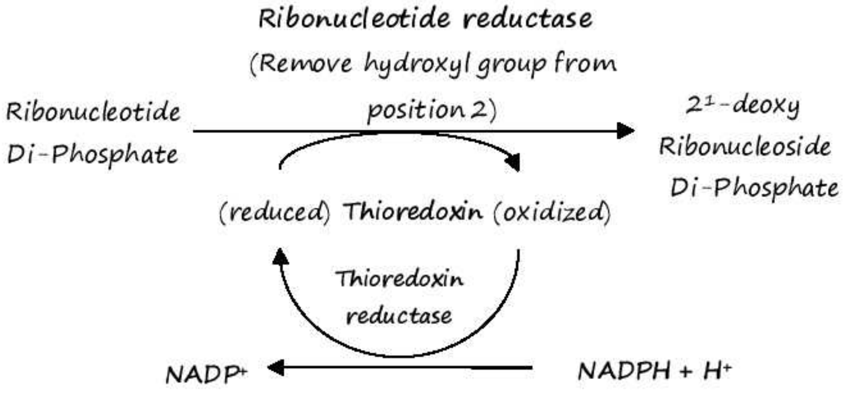
- When lot of free energy available: CTP


Glutamate
Without any enzyme
UTP

- It is a type of DNA damage

Conversion of Ribose nucleotide to Deoxy-Ribose nucleotide 00:18:28

- UDP → dUDP
- Occur only when cell undergo DNA synthesis


Ribonucleotide reductase
(Remove hydroxyl group from position 2)
Ribonucleotide Di-Phosphate
2¹-deoxy Ribonucleoside Di-Phosphate
(reduced) Thioredoxin (oxidized)
Thioredoxin reductase
NADP+
NADPH + H+

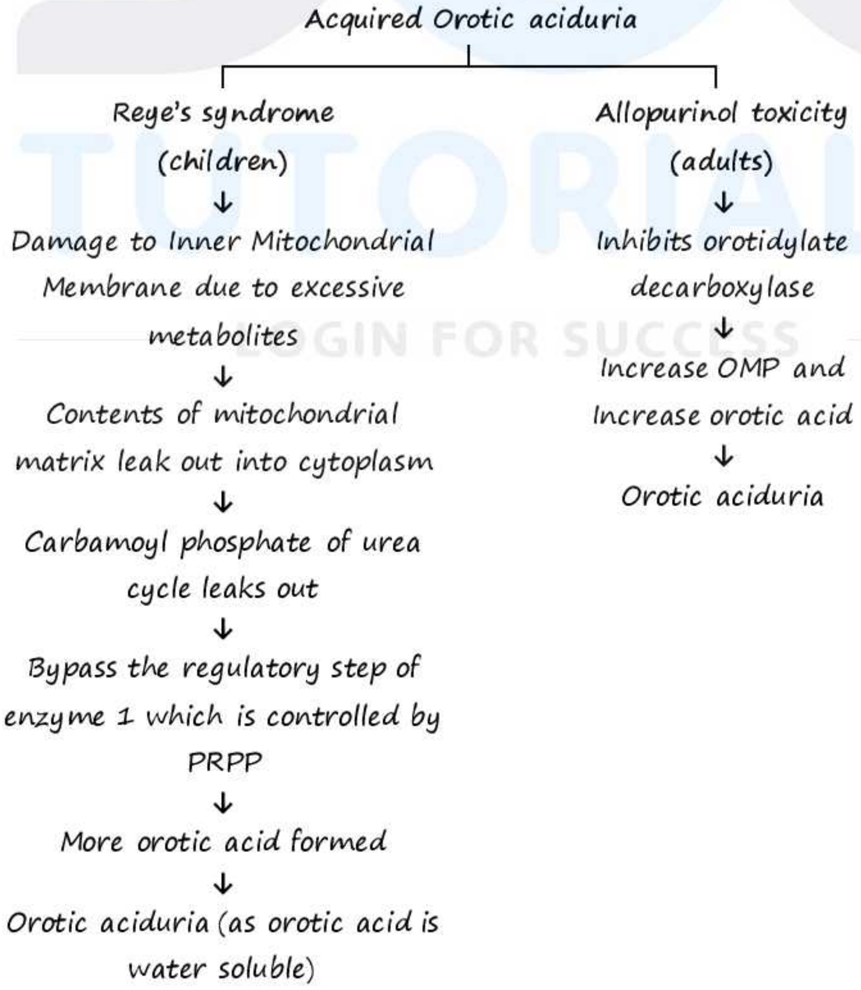
- Thioredoxin reductase contains 21st aminoacid (selenocysteine)

Pyrimidine degradation 00:21:28

- End products – freely water soluble
  - Ammonia (mnemonic: AB_2C)
  - β-alanine
  - β-aminoisobutyrate
  - Carbondioxide

Inherited disorders associated with pyrimidine metabolism 00:22:41

- β hydroxy butyric aciduria
- Orotic aciduria (Type I and II)



Acquired Orotic aciduria

Reye's syndrome (children)
↓
Damage to Inner Mitochondrial Membrane due to excessive metabolites
↓
Contents of mitochondrial matrix leak out into cytoplasm
↓
Carbamoyl phosphate of urea cycle leaks out
↓
Bypass the regulatory step of enzyme 1 which is controlled by PRPP
↓
More orotic acid formed
↓
Orotic aciduria (as orotic acid is water soluble)

Allopurinol toxicity (adults)
↓
Inhibits orotidylate decarboxylase
↓
Increase OMP and Increase orotic acid
↓
Orotic aciduria

Urea cycle disorders

- Orotic aciduria (due to leak of accumulated carbamoyl phosphate)
- Pyrimidine metabolism is absolutely normal

PRPP (Phospho Ribosyl Pyro Phosphate) role

- Denovo purine synthesis
- Salvage pathway of purines
- Pyrimidine synthesis
- NAD^+/ $NADP^+$ synthesis

DNA POLYMERASE

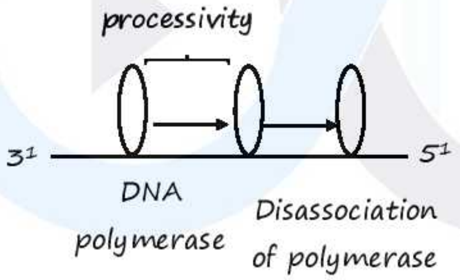
Properties of DNA Polymerase 00:01:03

i. Polymerisation

Polymerisation

Chain elongation
↓
- Rate of work/activity
- Nucleotides added per unit time (Nt/second)

Processivity
↓
- Amount of work done
- Total number of nucleotides added by a polymerase while it is attached to DNA during the process of replication



- For a best DNA polymerase – chain elongation and processivity should be high.

ii. Proof reading activity: 2 steps

Exonuclease function
↓ Followed by
Polymerase function

- Exonuclease function
  - $3^1 \rightarrow 5^1$ exonuclease function → present in all polymerase
  - $5^1 \rightarrow 3^1$ exonuclease function [removes the primer which attaches at 5^1 end]
    - Prokaryotes → DNA pol – I
    - Eukaryotes → DNA pol – β

- DNA pol-I and DNA pol-β have both $3^1 \rightarrow 5^1$ and $5^1 \rightarrow 3^1$ exonuclease activity

DNA Polymerase – I 00:08:25

- 1^{st} polymerase to be discovered in prokaryotes
- 3 functional domains

  1.polymerase

  2.$3^1 \rightarrow 5^1$exonuclease

  3.$5^1 \rightarrow 3^1$exonuclease
- By certain enzymes → DNA pol -I can be cleaved into 2 parts.

↓

Klenow fragment (DNA pol-I without $5^1 \rightarrow 3^1$exonulcease activity)

↓

Important role in genetic engineering

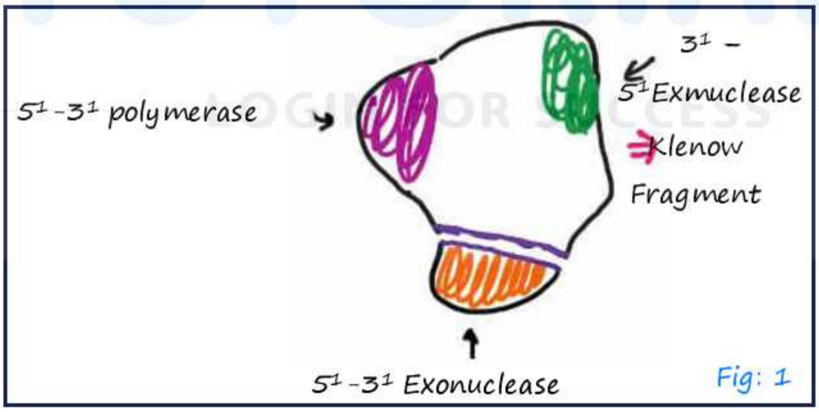

5^1-3^1 polymerase
3^1 – 5^1Exmuclease
Klenow Fragment
5^1-3^1 Exonuclease

Fig: 1

Types of DNA Polymerase: 00:11:54

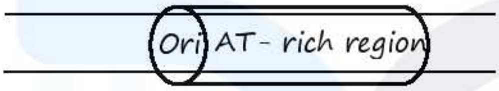
Prokaryotes	**Function**	**Eukaryotes**
DNA polymerase – I	Replaces the primer DNA repair	DNA polymerase β (beta)
DNA polymerase – II	DNA proof reading and repair	-
DNA polymerase III	**Processive polymerization** i. On both leading and lagging strands ii. On leading strand by DNA pol E - On lagging strand by DNA pol δ	DNA polymerase E (epsilon) DNA polymerase δ (Delta)
–	Mitochondrial DNA polymerase	DNA polymerase γ (gamma) [poor proof reading activity]
dna G (independent protein)	Primase synthesis of primer (RNA polymerase)	subunit of DNA polymerase α

- Note: DNA polymerases in eukaryotes as a part of complexes have a role in DNA repair function.

DNA SYNTHESIS/DNA REPLICATION

DNA Synthesis/Replication 00:00:46

- 'S' phase of cell cycle
- Separation of strands
  - Occurs at AT-rich region as it is simpler and easier to break due to weaker strength
    i. A=T, 2 hydrogen bonds
    ii. C = G, 3 hydrogen bonds



  - Ori (origin of replication) – includes both strands
  - Number of Ori
    i. Prokaryotes – 1
    ii. Eukaryotes – multiples (depends on length of DNA)
- Entry and attachment of ORE/OBP to Ori

  ↓ (Ori response elements/Ori Binding Proteins)

  Causes of denaturation of Ori region

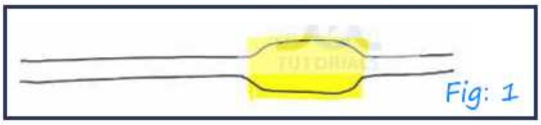


Fig: 1

  - Stablization of separated DNA strands by ssBPs

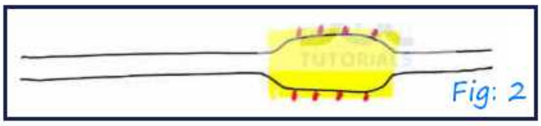


Fig: 2

ssBPs (Single stranded binding proteins) – prevent premature reannealing of DNA strands

  - Further separation of DNA by Helicase

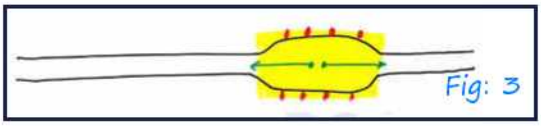


Fig: 3

A pair of helicase simultaneously separates the strands in both directions

↓

Unwinding of DNA

↓

Supercoiling (due to torsional strain)

- Topoisomerases – relives the torsional strain there by prevents supercoiling

- Note:
  - Can't initiate polymerization
  - Only elongates the existing nucleic acid

- RNA polymerase (primase) → forms initial nucleic acid i.e. RNA (primer)

↓

Primer extended by

DNA pol-III (Prokaryotes)

DNA pol δ and E (Eukaryotes)

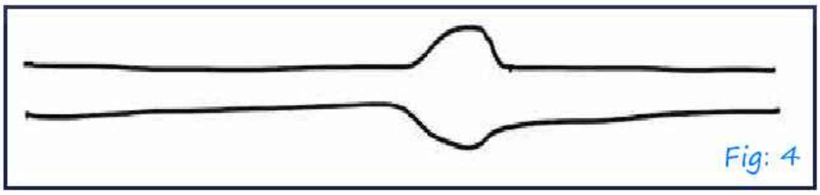


Fig: 4

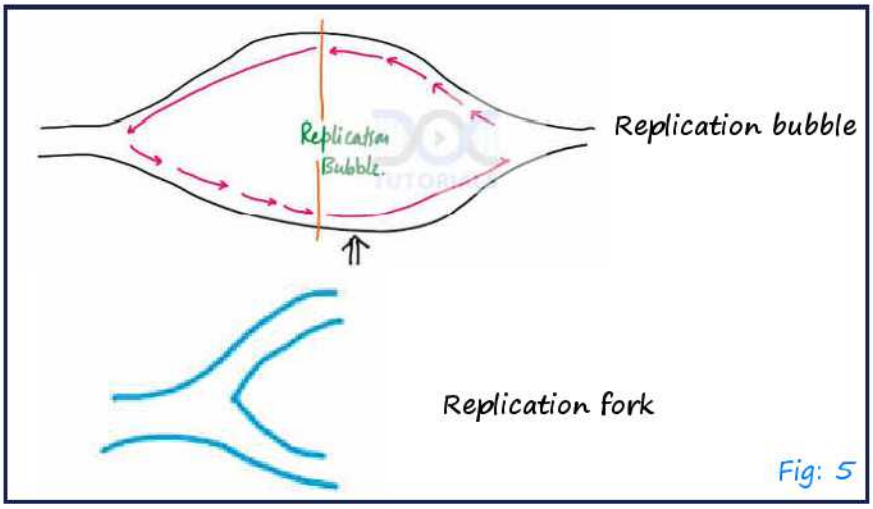

Replication Bubble.
Replication bubble
Replication fork

Fig: 5

- Replication fork:

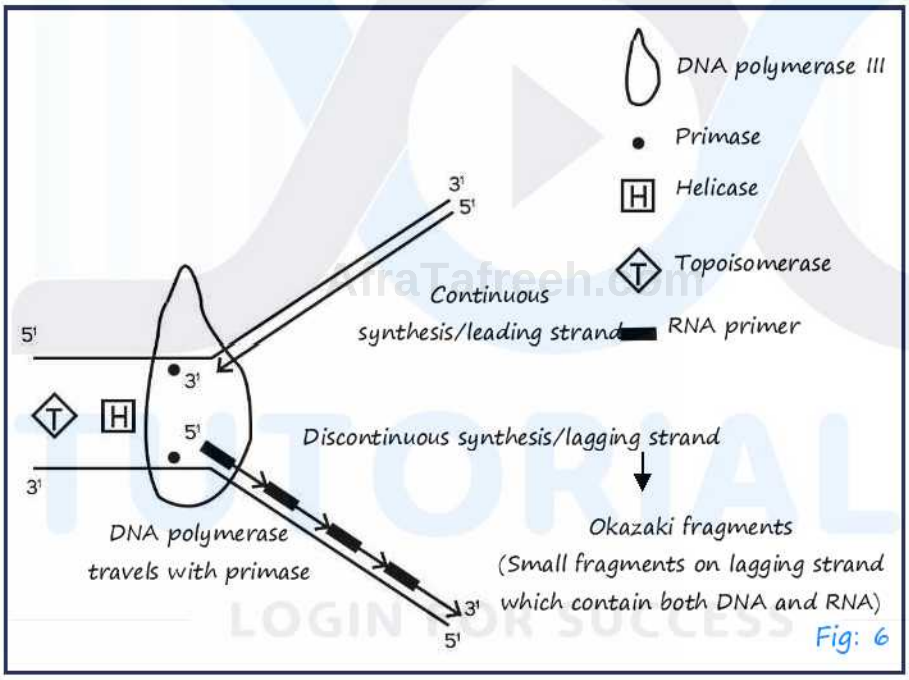

DNA polymerase III
Primase
Helicase
Topoisomerase
RNA primer
3'
5'
Continuous
synthesis/leading strand
5'
3'
T
H
5'
3'
Discontinuous synthesis/lagging strand
Okazaki fragments
(Small fragments on lagging strand
which contain both DNA and RNA)
DNA polymerase
travels with primase
3'
5'

Fig: 6

DNA Polymerase I replaces the primers on lagging strand: 00:15:47

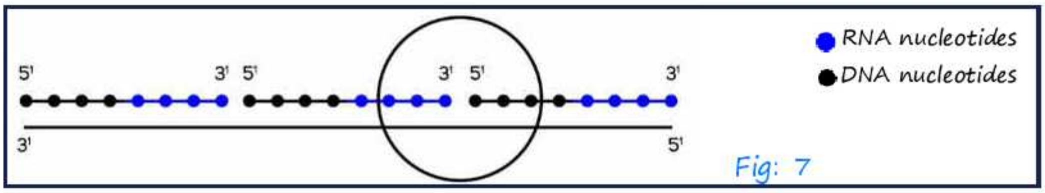

RNA nucleotides
DNA nucleotides
5'
3'
5'
3'
5'
3'
3'
5'

Fig: 7

Entry of DNA pol – I

↓

$5^1 \rightarrow 3^1$ exonuclease activity of DNA pol – I
(removes nucleotides from 5^1 end)

↓

Removes the ribonucleotide at 5^1 end i.e the primer

↓

Replace with deoxyribonucleotide (due to polymerase activity)

↓

The new nucleotide only be added at 3^1 end

↓

So, the next okazaki fragment is extended

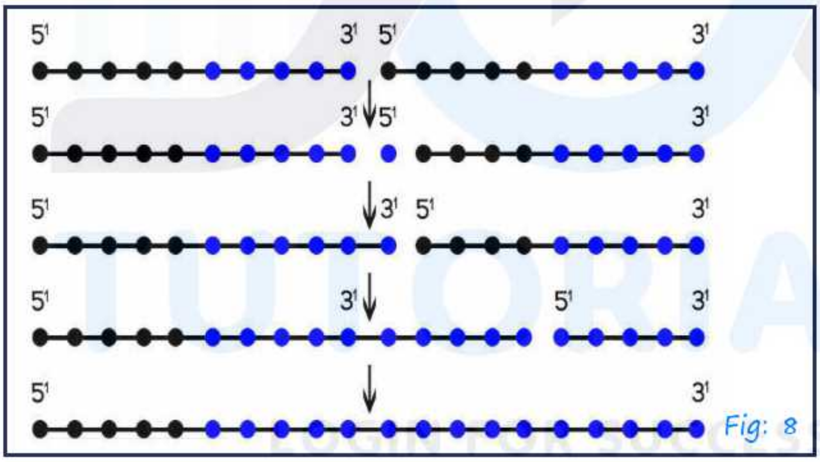


Fig: 8

Removal and replacement of ribo nucleotide

New nucleotide added at 3^1 end and next okazaki fragment extended

Removal of whole primer but 2 okazaki fragments are not joined

Joined by DNA ligase

- Last okazaki fragment

  ↓

  RNA primer degraded due to short ½ life of RNA

  ↓

  Because of no okazaki fragment behind

  ↓

  no extension by DNA

  ↓

  i.e. no replacement of the primer of last okazaki fragment/last primer can't be replaced

  ↓

  Shortening of telomere

  (i.e. The new DNA strand is shorter than parent DNA strand)

- Series of replication/cell division

  ↓

  Telomere exhausts

  ↓

  Apoptosis of cell

  ↓

  Prevented by hayflick's limit

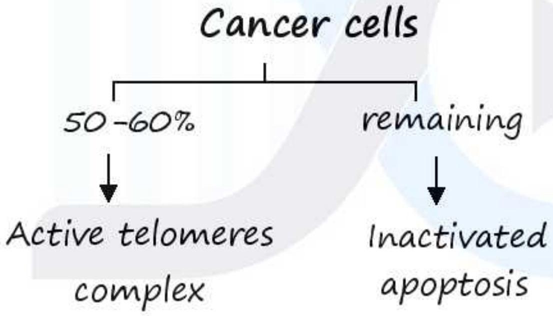
Hayflick's Limit 00:21:40

- Fixed number or limit to the number of times a cell can divide in the body (in-vivo)
- Dependent on length of telomere and depends on species
- It is true for somatic cells but not true for cell culture
- For human somatic cells →~ 70 divisions

- Hair
  - i. width – 20 μ
  - ii. 1 mm long – 50 cells
    - 1 cm long – 500 cells
    - 10 cm long – 5000 cells

Telomerase complex 00:24:13

- Mechanism to bypass Hayflick's limit
- It regenerates telomere
- Active in hematopoietic stem cell, hair follicle, epithelium of skin and gut, male germ layer, some cancer cells


Cancer cells
50-60%
remaining
Active telomeres complex
Inactivated apoptosis

- Once replication completes → DNA rearranges as chromatin

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Proteins and Enzymes in Replication 00:27:25

ORE/OBP	Denaturation of Ori
ssBPs	Prevent premature reannealing
Helicase	Unwinding of DNA
Topoisomerase	Relieves torsional strain
Primase	Synthesis of RNA primer

DNA pol – III (prokaryotes)	Polymerisation
DNA pol – I	Replacement of primer on lagging strand
DNA ligase	Joins okazaki fragments

- Telomerase
  - Not critical for replication
  - It is required in cells that divide unlimited times
- Blockage of replication
  - i. Nucleotide analogues – inhibits extension
  - ii. Chemotherapy (5-FU, Methorexate) – prevents formation of substrates i.e. deoxy nucleotides

Fate of primer on leading strand: 00:31:40

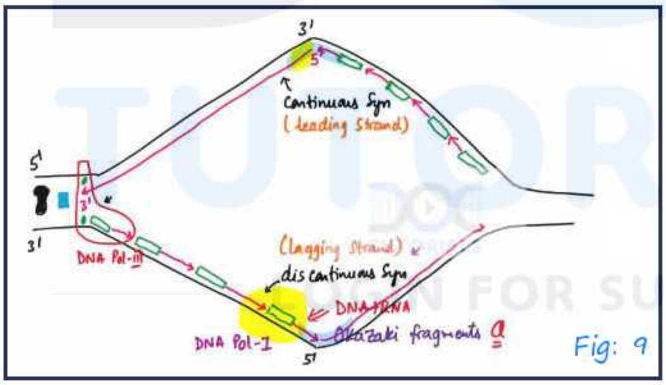

3'
5'
Continuous Syn
(leading Strand)
5'
3'
3'
3'
DNA Pol-III
(lagging Strand)
discontinuous Syn
DNA-RNA
Okazaki fragments
DNA Pol-I
5'

Fig: 9

- The 1st okazaki fragment of lagging strand replaces the primer of leading strand which is on opposite side of replication fork.

DNA DAMAGE AND REPAIR

Introduction 00:00:40

- DNA polymerase – adds nucleotides by chargaff's rule
  - error rate without proof reading activity → 1 in 10^6 nucleotides
  - error rate with proof reading activity → 1 in 10^8 nucleotides
- Number of base pairs to be replicated in a human cell → 3.1×10^9 base pairs
- After replication = $\frac{3.1 \times 10^9}{10^8}$ → 31 errors in each cell after every replication
- Causes of DNA damage – Radiation
  - Chemical reactions
  - Oxidative stress
- If DNA damage not repaired
  - mutation → disease/malignancy
  - apoptosis → loss of tissue function

DOI TUTORIALS LOGIN FOR SUCCESS

Types of DNA damage: 00:03:20

i. single base damage
  - examples mismatch
  - spontaneous deamination (C→ U)

ii. Two bases damage → example thymidine dimers (Form on exposure to UV light)

iii. DNA breaks
  - Single stranded DNA break
  - Double stranded DNA break

iv. Cross linking

- Between different strands of DNA
- No efficient DNA repair mechanism

Type of DNA damage	Associated repair mechanism	Specific disease when DNA repair mechanism is defective
i. Single base - Mismatch	Mismatch repair (MMR)	Lynch syndrome/HNPCC (Hereditary Non-polyposis colorectal cancer)
- Spontaneous deamination	Base Excision Repair (BER)	Mut Y Homologue Associated polyposis (MAP) – subtype of FAP (Familial Adenomatous Polyposis)
ii. Two bases - Thymidine dimers	Nucleotide Excision Repair (NER)	Xeroderma pigmentosa cockayne syndrome Trichothiodystrophy
iii. DNA breaks - ssDNA break	Homologous repair (HR)	Bloom syndrome Werner syndrome BRCA 1, 2 gene defect
- ds DNA break	Non – Homologous end joining (NHEJ)	SCID Radiation sensitive SCID (Severe Combined Immuno Deficiency)

	Photosensitivity	Skin lesions	Skin cancer
Xeroderma pigmentosa (Most common and severe)	✓	✓	Higher chance
Cockayne syndrome	✓	✓	Moderate chance
Trichothiodystrophy	✓	✓	Less chance

- Only BRCA 1 or BRCA 2 defective → breast cancer
- Both BRCA 1 and BRCA 2 defective
  - male → breast, prostate cancer
  - female → breast, ovarian cancer

DNA SYNTHESIS (TRANSCRIPTION)

In prokaryotes 00:00:40

- Single type of RNA polymerase
- RNA polymerase – 5 subunits (2α, β, β', ω)
- Transcriptionally active
- RNA polymerase (+) σ (Sigma) subunit
- σ Sub unit gives information about where transcription has to start
- σ Subunit identifies promoter
- For different promoters there are different σ subunits (σ n) (n → Promoter for which σ is specific)

Steps of transcription:

- Initiation
- Elongation
- Termination

Initiation

Once σ subunit attaches to RNA polymerase

↓

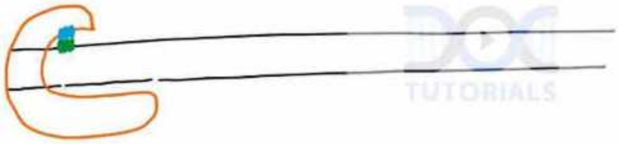
RNA polymerase Holoenzyme

↓

Translocate to DNA

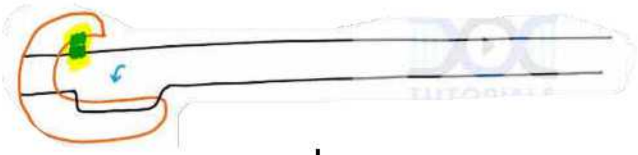
↓

Attaches to the promoter segment which is identified by σ subunit



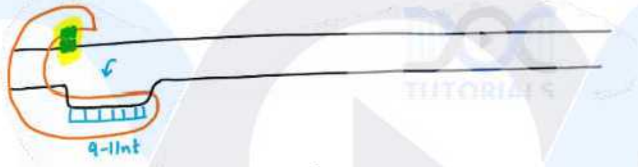
↓

- RNA polymerase denatures the overlapping DNA segment (RNA polymerase holoenzyme attached to denatured DNA is known as pre-initiation complex of transcription)



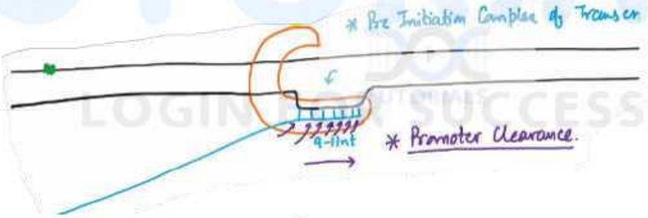
↓

- Small segment of RNA (9 to 11 nucleotides) is transcribed which is complementary to the denatured template DNA strand



↓

- Promoter clearance – last step of initiation RNA polymerase moves away from promoter as the formed nucleotides provide foot hold for RNA polymerase to start moving



↓

- As RNA polymerase keeps moving forward, the template strand is used to elongate the RNA
→Elongation

Note:

- σ subunit dissociates
  i. During elongation
  ii. After termination

Termination

- Rho dependent/ATP dependent
- Rho independent/ATP independent

Rho/ATP dependent

- More common
- Condition to be satisfied → Transcription of Rho-Binding site on RNA

Mechanism:

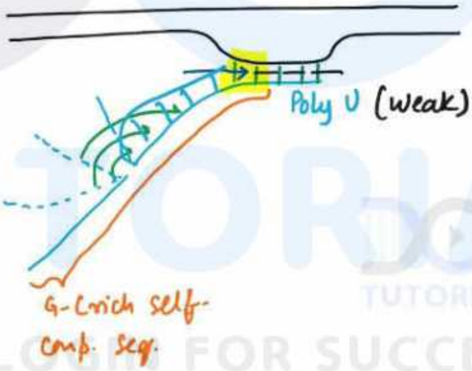
Transcription of Rho binding site
↓
Rho subunit attaches
↓
Denatures the attachment between DNA and RNA (ATP required)
↓
RNA dissociates from DNA
↓
RNA polymerase released
↓
Termination

Rho/ATP independent

- 2 conditions to be satisfied
  1. G-C rich self-complementary sequence should be available in RNA transcript
  2. Followed by poly U segment (So the corresponding template DNA strand consists of A, A=U → easy to separate as it is weak)

Mechanism:

G-C rich self-complementary sequence
(Extends to the transcript part which is attached to DNA)
↓
Start to pair up
↓
RNA folds
↓
Strain at the site of attachment of RNA and DNA
↓
Poly U segment (Weak) cannot bear the strain
↓
RNA dissociates from DNA
↓
RNA polymerase released
↓
Termination



Eukaryotes: 00:16:13

- No σ subunits
- Transcription factors

  1. I_{ABCD}
  2. II_{ABCD}
  3. III_{ABCD}

- Transcription from II_D→ Most significant
- Composition of transcription factor II_D

  - TBP (TATA Box Binding Protein)
- TAF (Tata box binding protein associated factors)

Inhibitors of transcription: 00:18:43

Prokaryotes

By binding to β subunit of RNA polymerase

EX:

Rifampicin
Rifamycin
Streptovaricin
Streptoglygidin
Heparin

By binding to DNA template

EX:

Actinomycin
D/Dactinomycin

Eukaryotes:

- Alpha-Amanitin
- Obtained from mushroom (Amanita phyllodes)
- Mainly targets RNA polymerase III

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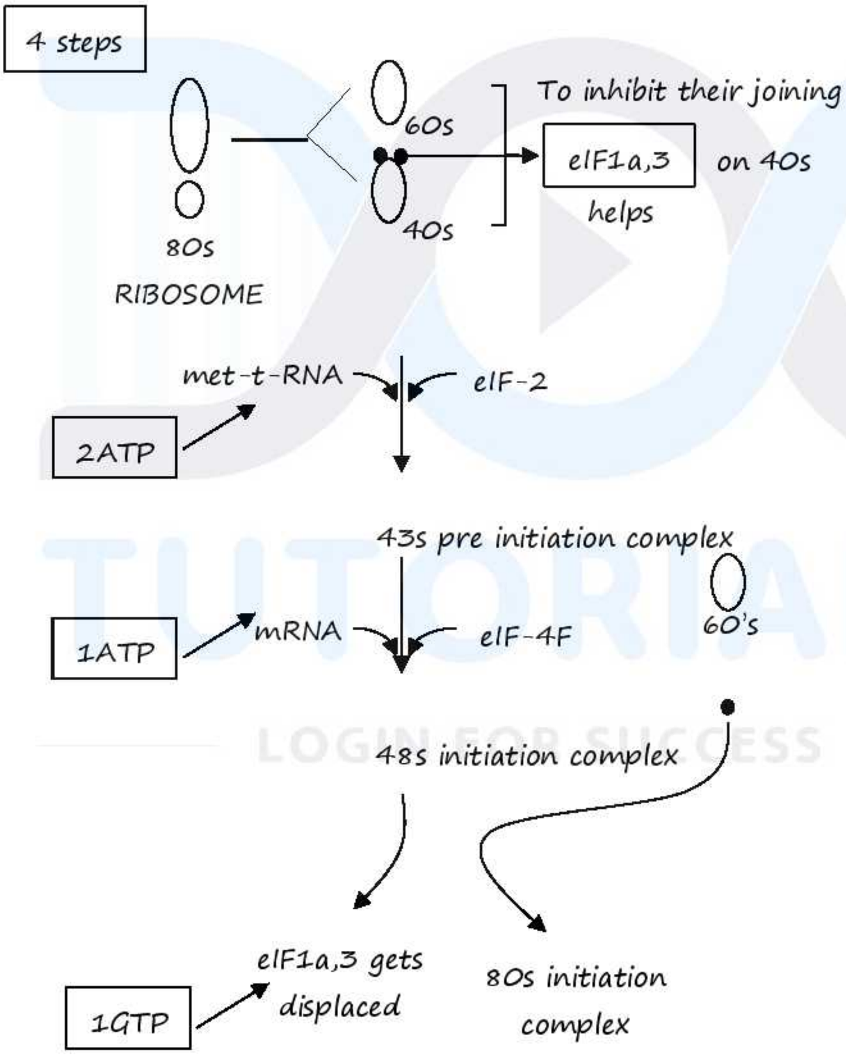
DNA SYNTHESIS (TRANSLATION) PART-II

- In eukaryotes

  1. Initiation
  2. Elongation
  3. Termination

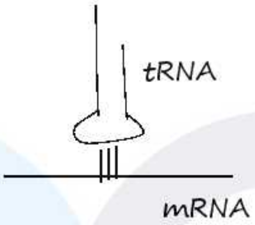
Initiation


4 steps
80s
RIBOSOME
60s
40s
To inhibit their joining
eIF1a,3
on 40s
helps
met-t-RNA
eIF-2
2ATP
43s pre initiation complex
60's
1ATP
mRNA
eIF-4F
48s initiation complex
1GTP
eIF1a,3 gets displaced
80s initiation complex

- eIF-2
  - α→Phosphorylation of α subunit leads to inactivation

  - β
  - γ
- eIF–4F
  - 4E→help identification of mRNA, phosphorylation of 4E leads to activation
  - 4A,4G

When tRNA & mRNA have to pair up



1GTP is required

- The process of initiation requires 4ATP equivalents (or) 3ATP + 1GTP

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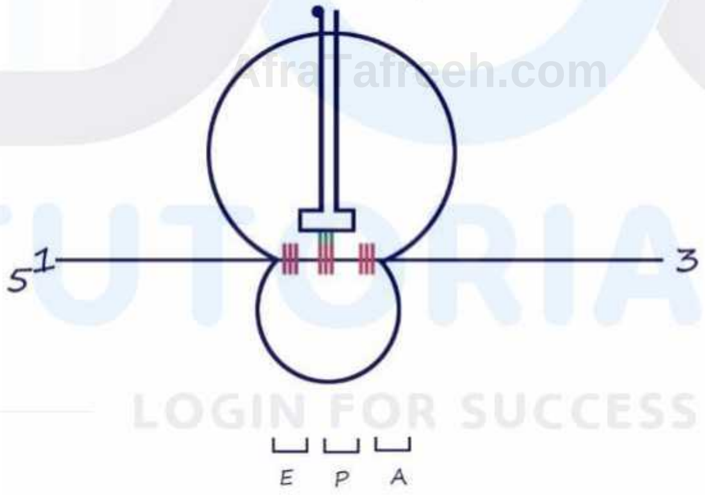


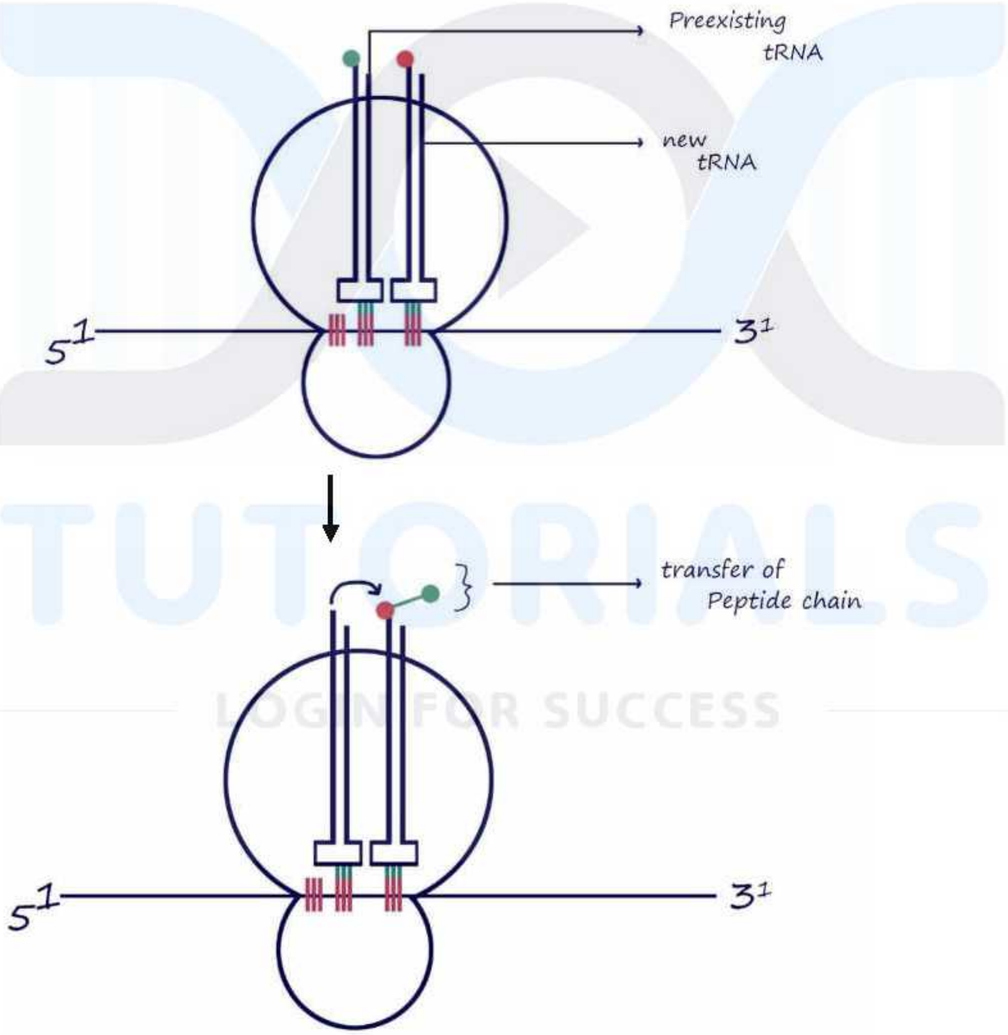
Ejector site	Peptide site	Acceptor site
↓	↓	↓
Any tRNA positioned at this site is displaced unstable	Methionyl tRNA positioned at this site	New incoming amino acyl tRNA gets attached here

Named relative to ribosomal position

AUG in case of 80s initiation complex

Elongation

- Entry of amino acyl tRNA (2ATP) required
- Attachment of amino acyl tRNA (1GTP) required
- Peptide bond formation (no ATP) – with help of peptidyl transferase
- Translocation (1GTP required)
- Ejection of empty tRNA (no ATP)


Preexisting tRNA
new tRNA
5'
3'
transfer of Peptide chain
5'
3'

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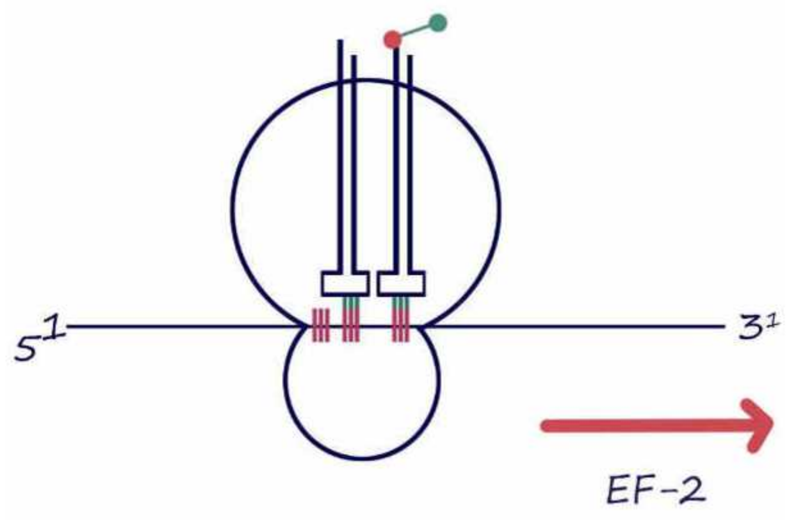
- After peptide bond formation completes, elongation factor-2

↓

It pulls ribosome forward by 1 codon



- Since, tRNA enters E site, gets ejected; new acceptor site is available

↓

Process continues till
A site is occupied by
stop codon (termination)
resulting in long peptide chain

Total ATP required for one round of elongation: 4ATP
for 1 aminoacids (or) 2ATP+2GTP

Note:

- For peptide bond formation → ATP is not required

Once the stop codon is there

Entry of 2 new molecules

Releasing factor RF 1,3/2,3
Has low affinity towards A site
(Usually as pairs)
Get attached only when A site
occupied by stop codon
occurs with help of petptidyl transferase

- At the end

Hydrolysis(1GTP/1ATP)
Occurs with help of peptidyl transferase
↓
Release of peptide chain from tRNA

Note:

- All 3 factors essential for termination

1. Stop codon
2. Releasing factor
3. Peptidyl transferase

Inhibition of translation

	In prokaryotes	In eukaryotes
	70s ribosome	80s ribosome
Splits into →	50s and 30s	60s and 40s
Peptidyl transferase →	23s RNA	28s RNA
	↑ ⊖	↑ ⊖
	as in antibiotics	by toxins

↓ reason why antibiotics can't act on our own cells; only targeting prokaryotes

In eukaryotes

60s	40s
28s rRNA 5.8s rRNA 5s rRNA 50 proteins – made here	18s rRNA 33-35 proteins

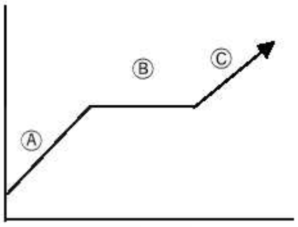
In prokaryote

50s	30s
23s rRNA 5s rRNA 33-35 proteins	16s rRNA 21 proteins

LAC OPERON

Lac Operon 00:00:57

- Regulatory model for gene expression
- Growth of bacteria that can metabolise both glucose and lactose when Lac operon is operative



- Ⓐ – Glucose metabolism, No Lactose metabolism
- Ⓑ – Glucose exhausted, no lactose metabolism
- Ⓒ – Lactose metabolism
- Lac operon consists of series of genes, that help to regulate and metabolize lactose

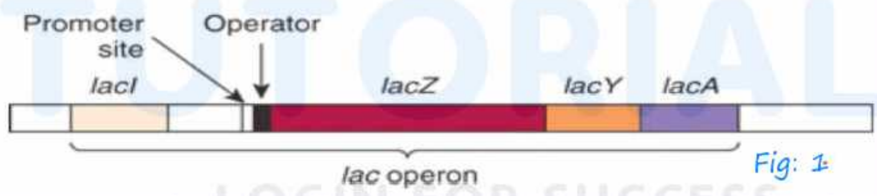
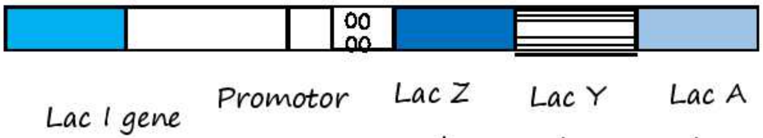


Fig: 1

Genes	Codes
Lac Z	B Galactosidase
Lac Y	Permease (transporter for lactose)
Lac A	Thiogalactoside transacetylase
Lac I	Lac operon repressor Protein

System Without Lactose 00:05:30



Lac Z, Lac Y, Lac A ↓ No Transcription

Lac I gene

↓

Transcription

↓

Translation

↓

Lac Operon
Repressor proteins

↓ Tetramerize

Tetramer ∞
∞

↓

Attach to operator which is
between promotor and Lac Z

↓

RNA Polymerase Cannot Approach promotor

↓

Prevents expression of Lac operon genes

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System with Lactose 00:08:10

Lactose has strong affinity for repressor proteins →
Lactose repressor

↓

No tetramer Formation

↓

Cannot block operator

↓

RNA Polymerase can approach Promotor site

↓

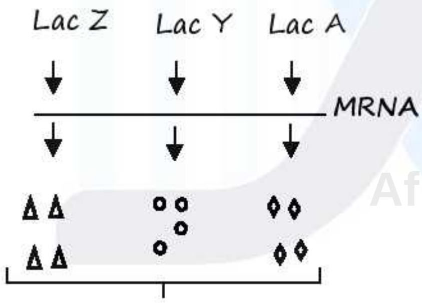
Expression of Lac operon genes

↓

Decreases glucose / decreases energy

↓
cAMP
↓
Binds to CAP
↓
Forms CAP – cAMP complex

- CAP – cAMP complex → Helps in attachment of RNA polymerase to promotor site
- Conditions for Lac operon genes to be expressed
  i. Lactose should be present
  ii. Glucose should be absent
- Lac genes expression:



Lactose metabolism occurs

- The 3 genes are transcribed on single mRNA
- But the proteins are translated separately (each protein has its start and stop codon)

Note:

1. Lac I repressor protein / Molecule
   - Negative regulator
   - Represses expression of lac operon
2. Lactose
   - Induces
   - Derepresses the Lac operon
   - Lactose analogues

- Same function as lactose
- Known as gratuitous inducers
- Example: IPTG (Iso Propyl ThioGalactoside)

3. CAP – cAMP regulator
   - Positive regulator
   - Required for optimal gene expression
4. Co-ordinate expression of 3 enzymes
   - Either all 3 enzymes will be expressed or none will be expressed
5. Each enzyme protein is translated separately
6. Once glucose is exhausted, there is lag phase before lactose metabolism starts because
   - cAMP synthesis
   - Transcription of genes

   Occur during lag phase
   - Translation of mRNA

Modified Lac Operon model

- Used in genetic engineering

Alpha – Complementation

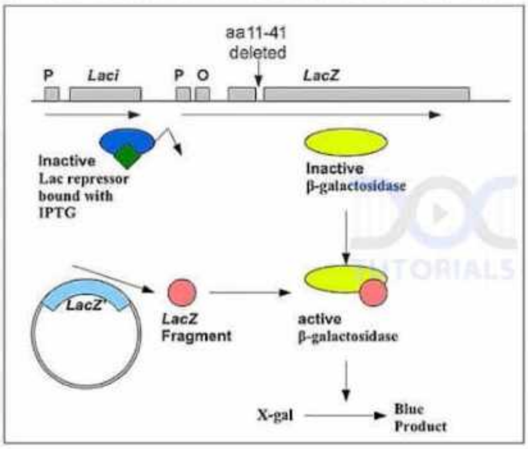

aa11-41
deleted
P
Laci
P
O
LacZ
Inactive
Lac repressor
bound with
IPTG
Inactive
β-galactosidase
LacZ'
LacZ
Fragment
active
β-galactosidase
X-gal
Blue
Product

Fig: 2

- Segment of Lac Z gene deleted and incorporated into a plasmid

- Alpha – complementation: Two protein which are inactive individually forms an active enzyme complex when they come together
- Segment selected from Lac Z gene has restriction site, so that restriction endonucleases can act and cleaves the DNA
- X gel (white) when metabolise by β galactosidase turns blue

Transformation

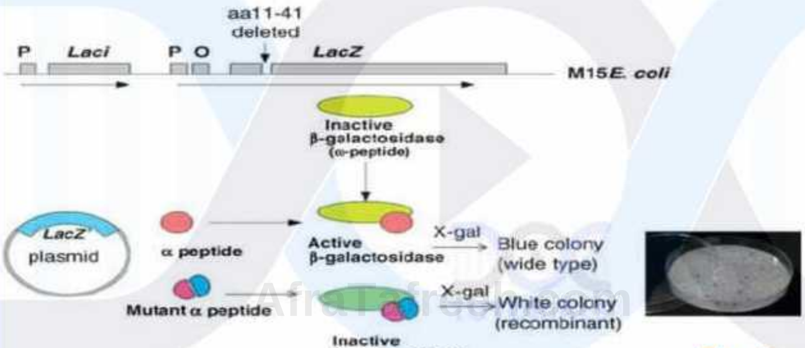
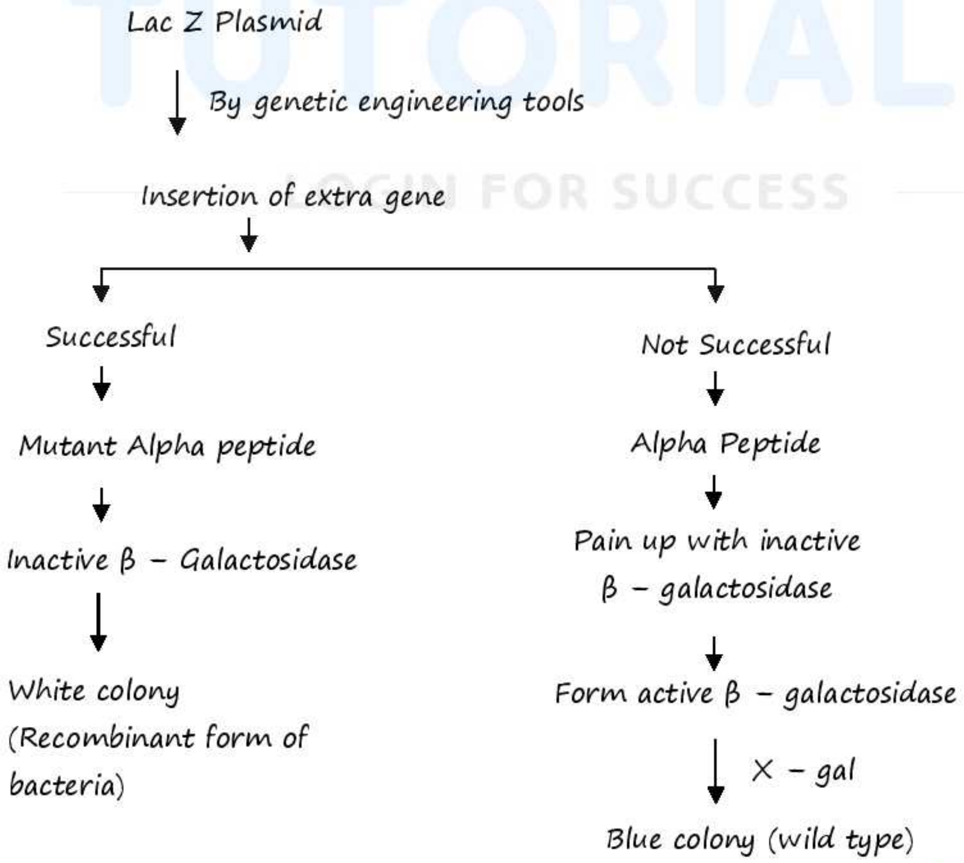

aa11-41
deleted
P
Laci
P
O
LacZ
M15E. coli
Inactive
β-galactosidase
(α-peptide)
LacZ'
plasmid
α peptide
Active
β-galactosidase
X-gal
Blue colony
(wide type)
Mutant α peptide
X-gal
White colony
(recombinant)
Inactive
β-galactosidase

Fig: 3


Lac Z Plasmid
By genetic engineering tools
Insertion of extra gene
Successful
Not Successful
Mutant Alpha peptide
Alpha Peptide
Inactive β – Galactosidase
Pain up with inactive
β – galactosidase
White colony
(Recombinant form of
bacteria)
Form active β – galactosidase
X – gal
Blue colony (wild type)

- This model of Lac operon used for genetic recombination
- Phenomenon by which colonies undergone recombination → Transformation
- Transformed colonies →
  - Extract the gene segment
  - Extract the product from that gene

POLYMERASE CHAIN REACTION (PCR)

PCR 00:00:47

- Diagnostic tool
- Research tool

Role – Identification of Presence & Absence of a know segment of DNA

Process 00:02:17

- Amplification of small segment of known DNA

Sample

↓

Extract DNA (RNA in case of virus with RNA genome)

↓

PCR for Unique gene (used in Infection) / Unique DNA Sequence (used in mutation)

- For every round of PCR, the number of copies of the known DNA segment double
- If 40 cycle done → number of copies = 2^{40} (~ millions of copies of DNA segment / amplicon)

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Critical Substrates required for PCR: 00:08:25

i. Template DNA

ii. Primers (pair) – to demarcate the beginning and the end of DNA segment

iii. DNA polymers (Taq polymerase – commonly used – Not critical substrate as other polymerases can also be used)

iv. Deoxynucleotides

v. Magnesium / manganese (Cofactors required by polymerase)

vi. Buffer – maintains optimum pH, so the enzyme can function

Steps of PCR 00:11:04

- Step 0: Design and synthesis of primers
- Step 1: Denaturation (separation of DNA strands) at 90° – 94°C for 15 – 45 sec
- Step 2: Annealing (joining of primers at target position) at 45° – 65° for 15 – 45 sec
- Step 3: Elongation / Extension of primer at 72° (for taq Polymerase) for 15 – 45 sec decreases after 30 – 40 cycles (1 cycle include step 1,2 and 3)
- Step 4: Final elongation

↓

Process of PCR completes

↓

DNA Gel Electrophoreses (Analyzes whether PCR is successful or not)

Modification in PCR 00:19:25

- Special dyes are used so that visualisation of result can occur simultaneously

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- Special dyes – Fluoresce only when attached to ds DNA
- Examples
  - SYBR Green (preferred)
  - SYTO 9
  - Eva Green
  - LC Green
- Ethidium bromide
  - Non-specific fluorescence (i.e., Fluoresce even when not attached to DNA)
  - Affinity for DNA
  - Used after gel electrophoresis
  - Carcinogenic

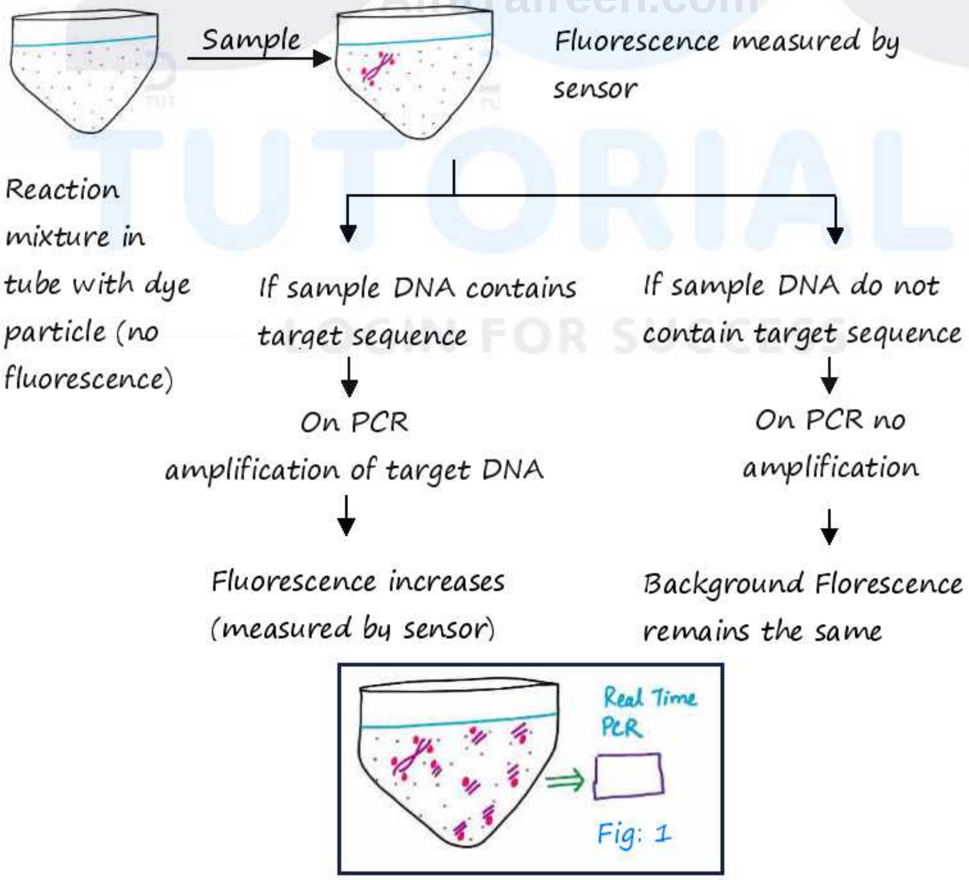
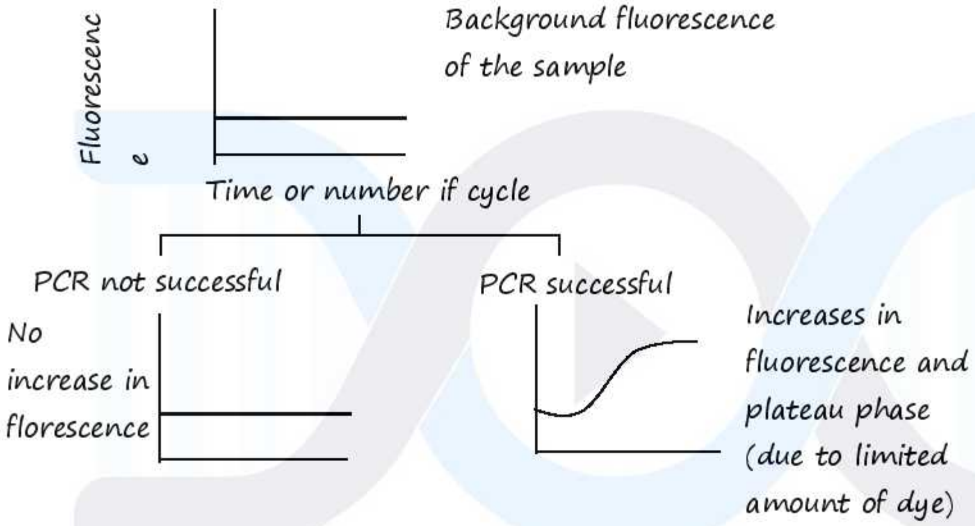

Sample
Fluorescence measured by sensor
Reaction mixture in tube with dye particle (no fluorescence)
If sample DNA contains target sequence
On PCR amplification of target DNA
Fluorescence increases (measured by sensor)
If sample DNA do not contain target sequence
On PCR no amplification
Background Florescence remains the same
Real Time PCR

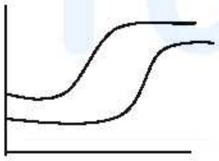
Fig: 1

Real time PCR:

- The increase in fluorescence can be measured in real time (i.e. Simultaneously as the PCR is progressing, we get to know whether PCR is successful or not, need not wait till the end of PCR)
- Fluorescence capture by Sensor is sent to computer which is represented as a graph



Control with sample:



- Calculating of the amount of DNA in starting unknown sample can be done by using controls
- So, the real time PCR can also be used for quantification i.e., measurement of relative quality of DNA present in given sample, hence real time PCR is also known as quantitative PCR (q – PCR)

Multiplex PCR

- Multiplex genes are targeted (provided sizes of amplicons are distinct)
- Example:

3 gene target segment of sizes 140 bp, 270bp, 490bp

↓

Primers for 3 segments used in same tube

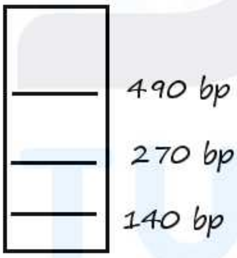
↓

PCR is rum together

↓

On gel electrophoresis

- 3 fragments separate out individually



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Nested PCR:

- Two sets of primers for an amplicon
- 2nd set of primer is present in product of 1st set primer
- 1st set of primer extended → 2nd set of primer extended
- Increases specificity of result

Note:

- Thermocycler → Based on peltier principle / Phenomenon
- PCR is done in it which can provide different temperatures rapidly as required

Isothermal PCR

- Performs all the 3 steps of PCR at same temperature
- Bst polymerase

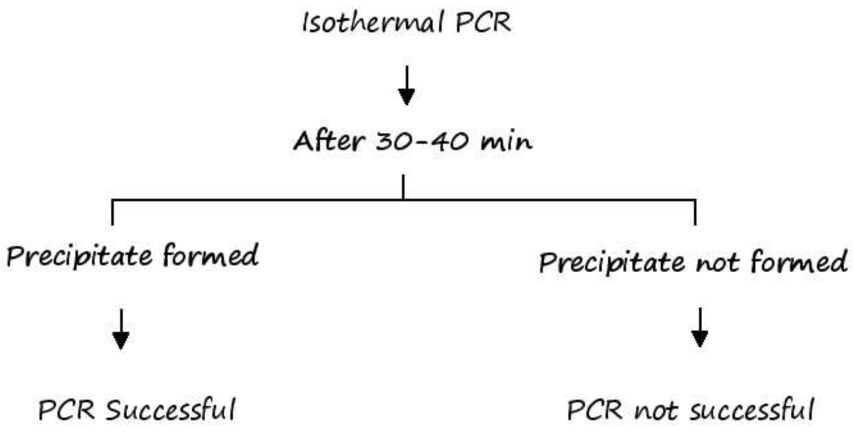
- Obtained from Bacillus stearothermophilus
- Polymerase function
- Helicase function

Optimum temperature for both function → 45° to 50° C

- Step 1: Denaturation by helicase at 45° to 50° C
- Step 2: Annealing
- Step 3: Elongation by polymerase

All type at same temperature

- PCR by Bst polymerase Complex completes by 30 – 40 min
- No need of DNA Gel Electrophoresis, because the rapid PCR creates precipitation of pyrophosphate
- Do not require thermocycler, it can be performed in water bath


Isothermal PCR
After 30-40 min
Precipitate formed
Precipitate not formed
PCR Successful
PCR not successful

(If doubt, perform DNA gel Electrophoresis)

- If sample RNA (HIV or COVID -19)
  - Reverse Transcription PCR (RT – PCR) is done
  - RNA $\xrightarrow[\text{Transcriptase}]{\text{Reverse}}$ c DNA (complementary DNA) → PCR
- Note: RNA degrades on heating

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Technique for amplification of RNA

- NASBA (nucleic Acid Sequence Based Amplification)
- It is an isothermal RNA amplification technique
- 3 enzymes are required
  - Reverse transcriptase
  - RNase H
  - RNA Polymerase

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Note:

- R nase H – Degrades RNA only in double stranded configuration (DNA – RNA hybrid or ds RNA)

HEME SYNTHESIS AND PORPHYRIAS

- Heme – Found in heme proteins

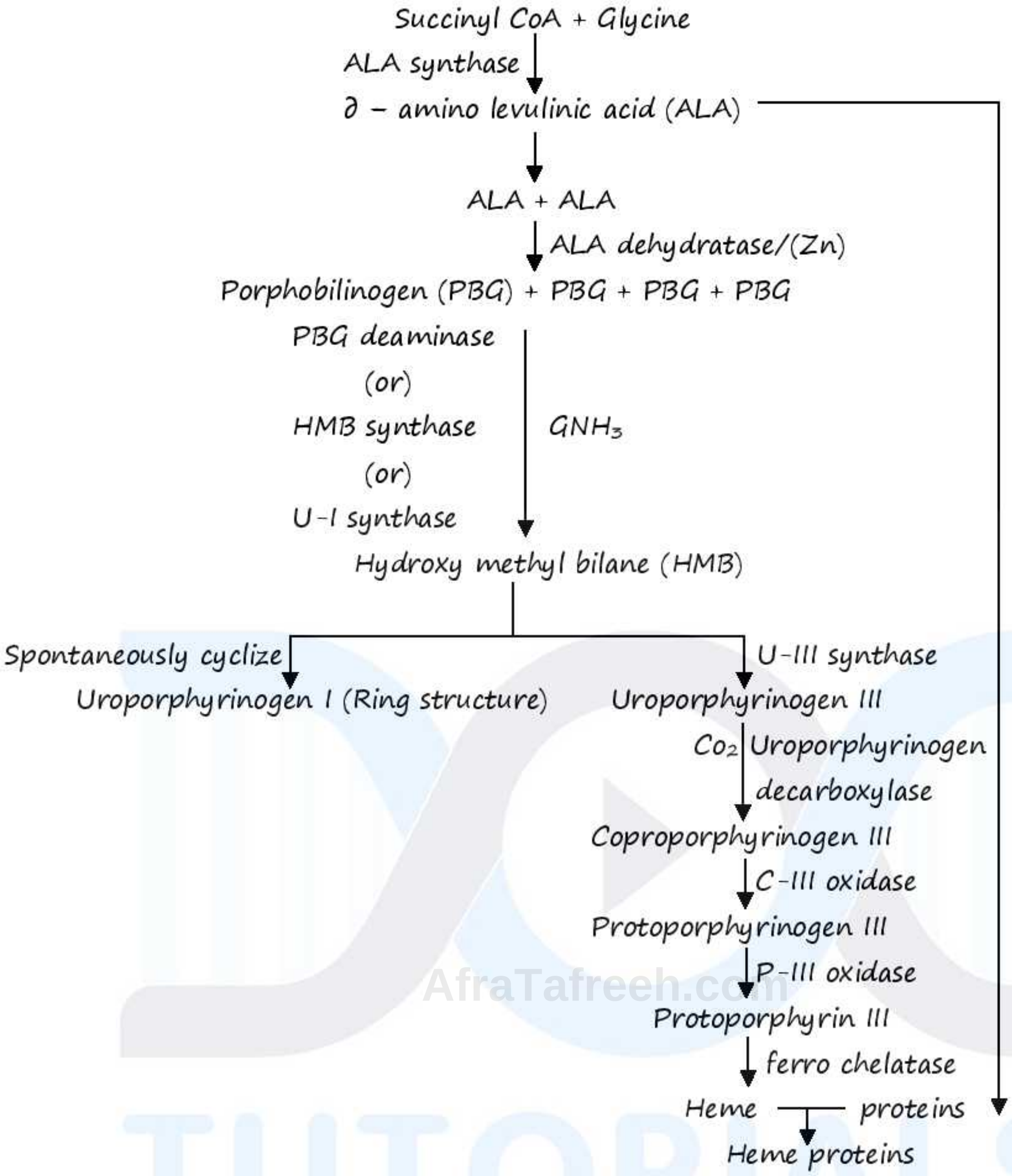
↓

Examples

1. Hemoglobin
2. Myoglobin
3. Cyt C
4. Cyt P450
5. Catalase
6. Tryptophan pyrrolase

Heme synthesis:

- Occurs in all tissues
- Maximum synthesis in liver & bone marrow
- Compartment – Mitochondria, cytoplasm
- Substances required – Succinyl CoA, Glycine, Fe^{+2} (ferrous iron)
- 1st enzyme – ALA synthase + Rate limiting enzyme + Targeted for feedback regulation


Succinyl CoA + Glycine
ALA synthase
∂ – amino levulinic acid (ALA)
ALA + ALA
ALA dehydratase/(Zn)
Porphobilinogen (PBG) + PBG + PBG + PBG
PBG deaminase
(or)
HMB synthase
(or)
U-I synthase
GNH₃
Hydroxy methyl bilane (HMB)
Spontaneously cyclize
Uroporphyrinogen I (Ring structure)
U-III synthase
Uroporphyrinogen III
Co₂
Uroporphyrinogen
decarboxylase
Coproporphyrinogen III
C-III oxidase
Protoporphyrinogen III
P-III oxidase
Protoporphyrin III
ferro chelatase
Heme
proteins
Heme proteins

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Note:

- ALA dehydratase – ZN containing metalloenzyme + It can be (–) by lead

Disorders of heme synthesis:

Deficiency of	Causes
ALA synthase	Anemia
ALA dehydratase	ALA dehydratase deficiency
PBG deaminase	Acute intermittent porphyria (No photosensitivity because of no ring structures formed)
U-III synthase	Congenital erythropoietic porphyria (No abdominal pain due to no involvement of liver)
Uroporphyrinogen decarboxylase	Porphyria cutanea tarda
C-III oxidase	Hereditary coproporphyrin
P-III oxidase	Variegate porphyria
Heme synthase/Ferro chelatase	Protoporphyria (Type of erythropoietic porphyria) (No abdominal pain)

Porphyria's:

- Occurs when intermediates of heme synthesis accumulate due to deficiency of one or more enzymes
- Previously diagnosis by
  - Urinary metabolites
  - Ehrlich's aldehyde test
  - Identifies +/- of PBG
  - Do not tell about type of porphyria
- Presently diagnosis by HPLC (High performance liquid chromatography)

- Tells about the type of porphyria depending upon metabolites getting accumulated in the serum
- Soret band: Peak in absorption at 400-410nm seen in patients of porphyria during an episode of porphyria due to presence of porphyrins

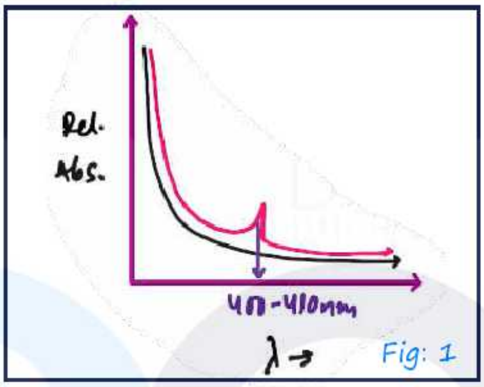
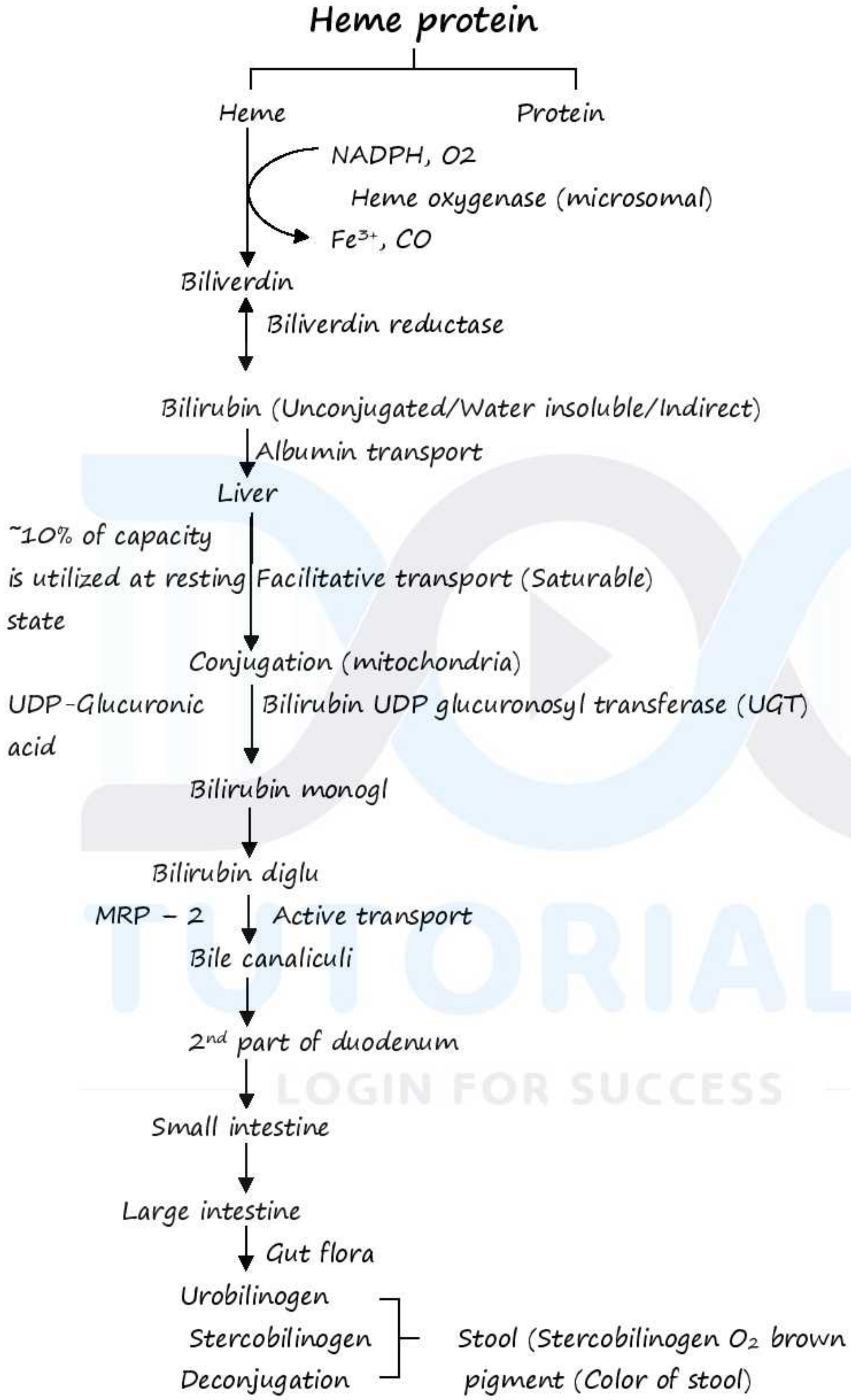


Fig: 1

Classical clinical presentation:

- Abdominal pain (Exception: Congenital erythropoietic porphyria, protoporphyria
- Photosensitivity (Exception: Acute intermittent porphyria) (Due to ring structures which absorb light and re-emit)
- Neuropsychiatric complaints

HEME DEGRADATION



Heme protein

Heme

Protein

NADPH, O2

Heme oxygenase (microsomal)

Fe^{3+}, CO

Biliverdin

Biliverdin reductase

Bilirubin (Unconjugated/Water insoluble/Indirect)

Albumin transport

Liver

~10% of capacity is utilized at resting state

Facilitative transport (Saturable)

Conjugation (mitochondria)

UDP-Glucuronic acid

Bilirubin UDP glucuronosyl transferase (UGT)

Bilirubin monogl

Bilirubin diglu

MRP – 2

Active transport

Bile canaliculi

2nd part of duodenum

Small intestine

Large intestine

Gut flora

Urobilinogen

Stercobilinogen

Deconjugation

Stool (Stercobilinogen O_2 brown pigment (Color of stool)

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Albumin transport:

Attachment of bilirubin on albumin

High affinity site　　Low affinity site

↓

- Capacity of this site is saturated at 20-25mg%
- When bilirubin >20-25mg% it leads to kernicterus in premature neonates

Multi-drug resistance like protein – 2 (MRP-2)

- Belongs to the class – Multi specific organic anion transporter
- Belongs to super family – ATP binding cassette transporter

HYPERBILIRUBINEMIA

- Hyperbilirubinemia also known as jaundice

Type	Causes
•Pre-hepatic/Hemolytic jaundice	Hemolytic anemia Infections inducing hemolysis (malaria) Drugs Mismatched blood transfusion
•Hepatic jaundice	Hepatitis – viral/bacterial/chemical Alcoholic liver disease Cirrhosis of liver Carcinoma of liver
•Post hepatic/obstructive jaundice	Stone in common bile duct (CBD) Carcinoma head of pancreas Worm infestation in CBD (Chinese flukeworm) Biliary atresia Biliary stasis (pregnancy)

- Bilirubin
  - Blood
  - Urine
- Urobilinogen
  - Urine
  - Feces/stool
- Enzymes – blood

Serum Bilirubin

- Normal – Direct = 0.1-0.4 mg%
  Indirect = 0.2 – 0.7 mg%
- Pre-hepatic jaundice = Indirect increases
- Hepatic jaundice = Indirect increases
  Direct increases
- Post hepatic jaundice = Direct increase

Urine Bilirubin

- Normal – absent
- Prehepatic jaundice – absent
- Hepatic jaundice – Increase only if micro-obstruction present
- Post hepatic jaundice – + + dark yellow or mustard colored urine

Urine Urobilinogen

- Normal – 0-4 mg/24 hr
- Pre-hepatic jaundice – Increases

- Hepatic jaundice – Decreases if micro-obstruction present
- Post hepatic jaundice - Absent

Fecal Urobilinogen

- Normal 40-280 mg/24hr
- Pre-hepatic jaundice – Increases
- Hepatic jaundice – Decreases
- Post hepatic jaundice - Absent, Pale/clay-colored stools

Enzymes

- Normal –
- Pre-hepatic jaundice – LDH – II increases (Suggests hemolysis)
- Hepatic jaundice - Increases ALT, Increases AST
- Post hepatic jaundice – Increases ALP
  Increases 5^1 nucleotidase
  Increases GGT

- In hepatic jaundice, direct bilirubin increases due to inflammation

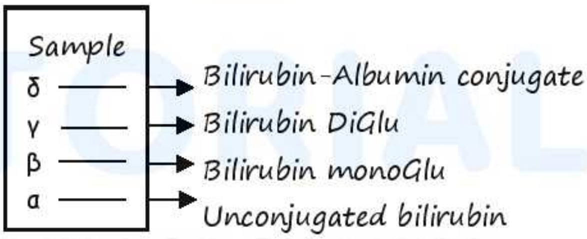
Inflammation
↓
Edema of hepatocytes
↓
Micro-obstruction of bile canaliculi
↓

Conjugated bilirubin before obstruction
↓
reflux into blood

- When jaundice is chronic, bilirubin attaches to albumin covalently

Treatment of underlying cause

- Bilirubin clears because of 24-36hrs t½
- If bilirubin does not comes down as expected
  ↓
  Identify whether elevated fragment is only bilirubin attached albumin or some other bilirubin
  ↓
  Done by electrophoresis for bilirubin


Sample
δ
γ
β
α
Bilirubin-Albumin conjugate
Bilirubin DiGlu
Bilirubin monoGlu
Unconjugated bilirubin

- If δ increases, bilirubin clears in 19-21 days (t ½ albumin)
- if other fragments increases → Re-investigate to find the cause of hyperbilirubinemia
- Reflux of bile → γ & β both seen in blood
- In bile canaliculi, CBD, intestine → γ only seen

Inherited Hyperbilirubinemia:

i. Unconjugated

- Criggler Najjar syndrome
  - Bilirubin UGT
  - I → 0%
  - II → < 5%

- Gilbert syndrome → <30%
- Lucey – Driscoll syndrome
  1. Also known as transient neonatal unconjugated hyperbilirubinemia
  2. Defect is not in neonate
  3. Mother produces mild inhibited of bilirubin UGT in breast milk
  4. Once conjugating system of newborn matures – jaundice disappears, no treatment required

ii. Conjugated

  a. Rotor Syndrome

  b. Dubin Johnson Syndrome